FINAL REPORT

Title:	Assessment of Pubertal Development and Thyroid Function in Juvenile Female CD® (Sprague-Dawley) Rats After Exposure to Selected Chemicals Administered by Gavage on Postnatal Days 22 to 42/43
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FINAL REPORT

Assessment of Pubertal Development and Thyroid Function in Juvenile Female CD® (Sprague-Dawley) Rats After Exposure to Selected Chemicals Administered by Gavage on Postnatal Days 22 to 42/43

ABSTRACT

The purpose of this study was to examine the sensitivity of pubertal assays to the effects of a wide variety of chemicals that are known to affect the endocrine system through different pathways and/or mechanisms of action. This assay detects agents that display anti-thyroid, estrogenic, anti-estrogenic [estrogen receptor (ER) or steroid enzyme mediated] activity, or alter luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin, growth hormone (GH) secretion or hypothalamic function. Therefore, EPA decided to test six chemicals that have various modes of action (i.e., atrazine, fenarimol, methoxychlor, bisphenol A, ketoconazole, and proplthiouracil), using the female pubertal assay study design.

The study was conducted in two components. In each component, F1 females, produced from undosed timed-pregnant CD® (Sprague-Dawley) rats (the F0 generation), were tested. On the day of birth [postnatal day (pnd 0)], F1 pups were counted, sexed, weighed, and examined externally. On pnd 4, the litters were standardized to ten pups, maximizing the number of female pups. Natural litters with ten or fewer pups were not culled. The F0 females were allowed to rear their pups to pnd 21. F1 survival, gender identification, gross observations, and body weight were recorded on pnd 4, 7, 14, and 21. On pnd 21, F1 females were weaned and weight ranked across litters, then randomized into the treatment groups based on body weight. Fifteen F1 females were assigned to each treatment group in each component. F1 females were orally dosed with a test compound or the vehicle (Mazola® corn oil) from pnd 22 to pnd 42/43. Dose volume (5 ml/kg/day) was based on daily body weight. In Component 1, animals received atrazine (75 or 150 mg/kg/day), fenarimol (50 or 250 mg/kg/day), or methoxychlor (25 or 50 mg/kg/day) in corn oil. In Component 2, animals received bisphenol A (400 or 600 mg/kg/day), ketoconazole (50 or 100 mg/kg/day), or propylthiouracil (2 or 25 mg/kg/day) in corn oil. A separate vehicle control group dosed with corn oil was run concurrently with each component. Body weights for the F1 females were recorded during the postweaning treatment period to scheduled sacrifice. Clinical signs were recorded twice daily during the treatment period. Beginning with pnd 22, F1 females were examined for vaginal patency. The day of complete vaginal patency was identified as the age of vaginal opening, and body weight was recorded on that day. Vaginal cytology was monitored daily from acquisition of vaginal opening to necropsy. At necropsy on pnd 42/43, females were euthanized and blood was collected by external cardiac puncture for analysis of thyroxine (T4) and thyroid-stimulating hormone (TSH). Body, liver, paired kidney, pituitary, paired adrenal, uterine and

paired ovarian weights were recorded, and the ovaries, uterus, and thyroid were evaluated histopathologically.

Observations

The following observations were made:

- **Atrazine.** Treatment with atrazine at 150 mg/kg/day delayed puberty, as evidenced by delayed vaginal opening. The postnatal day of vaginal opening exhibited a significant delay at the high dose (36.4 days vs. 32.9 days for the control group). No effect was observed at the low dose of atrazine although the data exhibited a linear decreasing trend. Body weight and body weight gain were decreased at both dose levels during treatment, although not at acquisition of vaginal opening. Both absolute and adjusted paired ovary weight exhibited a linear decreasing trend. However, only the high-dose absolute ovary weight was significantly different from the control value. There was no significant effect of treatment on uterine weight with or without fluid (absolute or adjusted for necropsy weight), circulating T4 or TSH levels, or histopathological changes in the thyroid, ovaries, or uterus.

- **Fenarimol.** Fenarimol at 50 and 250 mg/kg/day did not alter the day of acquisition of vaginal opening compared to the control group, although body weight at acquisition was decreased at the high dose. In addition, body weight and body weight change were decreased at the high dose during the treatment period and at sacrifice. There was no effect of treatment on absolute or adjusted thyroid weight. Circulating T4 levels were significantly reduced at the high dose, whereas an increase in circulating TSH levels was observed at 50 and 250 mg/kg/day. These effects on thyroid hormone levels were consistent with the observation of minimal follicular hypertrophy in 33% of the high-dose thyroids. Absolute uterine weight (without fluid) was significantly reduced at the high dose, but exhibited no treatment effect when adjusted for necropsy weight. No treatment-related histopathology of the uterus was observed. Absolute and adjusted paired ovary weight exhibited a decreasing trend, but the tissues exhibited no adverse histopathological effects.

- **Methoxychlor.** Methoxychlor at 50 mg/kg/day decreased body weight in the high dose during the last 6 days of dosing. In addition, body weight change for the treatment period was decreased at both the 25 and 50 mg/kg/day dose level. Accelerated vaginal opening was observed at both 25 and 50 mg/kg/day methoxychlor, in the presence of decreased body weight on the day of acquisition. Decreased adjusted paired ovary

weight was observed at the high dose. There was no treatment effect on absolute or any other adjusted organ weight, circulating thyroid hormone levels, or histopathology.

- **Bisphenol A.** Treatment with bisphenol A resulted in decreased body weight gain (pnd 22 to 42) and body weight at necropsy (pnd 42). The postnatal day of vaginal opening was not affected by bisphenol A treatment. Body weight of the high-dose animals was significantly reduced on the day of acquisition, compared to the controls. Absolute, but not adjusted uterus without fluid weight was decreased at the high dose. Absolute paired ovary weight was decreased at both doses, whereas adjusted paired ovary weight exhibited a decreasing trend. Ovarian hypoplasia was observed in 21% of the high dose animals. Circulating thyroid hormone levels were unaffected by treatment.

- **Ketoconazole.** The day of vaginal opening was not affected by ketoconazole treatment. Effects at the high dose included a decrease in absolute paired ovary and uterus (with or without fluid) weight. Adjusted liver and paired kidney weight was increased at both doses of ketoconazole. Ovarian pathology increased in incidence and severity with dose. Cytoplasmic vacuolization of the corpora lutea was observed in 12/15 and 9/15 of the low and high-dose animals, respectively. The five remaining animals in the high-dose group exhibited a complete absence of corpora lutea. Ketoconazole treatment did not adversely affect circulating thyroid hormone levels.

- **Propylthiouracil.** As expected, propylthiouracil produced a decrease in T4 and an increase in TSH circulating levels, increased adjusted thyroid weight, and thyroid follicular cell hypertrophy/hyperplasia at both 2 and 25 mg/kg/day. Acquisition of vaginal opening was not affected by propylthiouracil treatment. In addition, other than the thyroid, treatment with propylthiouracil did not affect adjusted organ weights.

OBJECTIVE

Purpose and Applicability

The purpose of this protocol is to outline procedures for the quantification of the effects of chemicals on pubertal development and thyroid function in the intact juvenile female rat. This assay detects agents that display anti-thyroid, estrogenic, anti-estrogenic [estrogen receptor (ER) or steroid enzyme mediated] activity, or alter luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin, growth hormone (GH) secretion or hypothalamic function.

Required Endpoints:

- Growth (daily body weight)
- Age and Weight at Vaginal Opening
- Serum thyroxin (T4) and thyroid stimulating hormone (TSH)
- Thyroid Histology
- Uterine and Ovarian Weights and Histology
- Vaginal cytology; histology to be conducted only where warranted.
- Liver, kidney, pituitary, and adrenal weights
- Onset of cyclicity (obtained from vaginal cytology)

To further define the procedures for this protocol, EPA tested six chemicals that have various modes of action:

- atrazine (affects the hypothalamus-pituitary axis in female rats and ovulation);
- fenarimol (a weak aromatase inhibitor);
- methoxychlor (a xeno-estrogen through α-estrogen receptor, anti-estrogen through β-estrogen receptor and an anti-androgen through androgen receptor mediated mechanism);
- bisphenol A (a weak environmental estrogen which binds to both the α and β estrogen receptor);
- ketoconazole (inhibits steroidogenesis in both sexes); and
- propylthiouracil (affects the thyroid directly, causing hypothyroidism).

The study was conducted in two components. Component 1 consisted of animals dosed with atrazine (75 or 150 mg/kg/day), fenarimol (50 or 250 mg/kg/day), or methoxychlor (25 or 50 mg/kg/day), and a concurrent vehicle control (corn oil) group. Component 2 consisted of animals receiving bisphenol A (400 or 600 mg/kg/day), ketoconazole (50 or 100 mg/kg/day), or propylthiouracil (2 or 25 mg/kg/day), and a concurrent vehicle control (corn oil) group.

MATERIALS AND METHODS

Test Materials and Dose Formulations

The test chemicals used in this study were procured and analyzed for purity by the Sponsor, as indicated below by gas chromotography with flame ionization detection (GC-FID), high pressure liquid chromotography (HPLC), or gravimetric methods. All bulk test chemicals were stored at room temperature. Test chemicals formulated in corn oil were stored at 4°C. Dose formulations were mixed in corn oil for administration at 5 ml/kg. One vehicle formulation was prepared to be administered to the control group animals concurrently with Component 1 compounds, whereas a separate vehicle formulation was prepared to be administered to the control group animals concurrently with Component 2 compounds. Formulations were prepared at Battelle, Sequim, and assayed between 90.5% and 112% of the target concentration prior to shipping to RTI International.

Animals and Husbandry

For Component 1, 20 timed-pregnant and 2 nonpregnant female outbred albino CD® (Sprague-Dawley) rats (Crl:CD®[SD] IGS BR) were received from Charles River Breeding Laboratories (Raleigh, NC) at gestational day (gd) 13. A separate order of 20 timed-pregnant and 2 nonpregnant female rats were received for use in Component 2. The females were 10 weeks old upon arrival. The F0 animals were individually housed during the quarantine period and during gestation, and with their litters during lactation in solid-bottom polycarbonate cages with stainless-steel wire lids (Laboratory Products, Rochelle Park, NJ) and Sani-Chip® cage litter (P.J. Murphy Forest Products Inc., Montville, NJ). Postwean, retained F1 females were housed under standard conditions until necropsy. All animals were housed in the RTI Animal Research Facility for the duration of the study. All animal rooms were on a 14:10 hour (light:dark) light cycle per day and were air-conditioned. Temperature (22±4°C) and relative humidity (30-70%) were continuously monitored, controlled, and recorded using an automatic system (Siebe/Barber-Colman Network 8000 System, Version 4.4.1, Loves Park, IL). Purina Certified Rodent Chow (No. 5002, PMI Feeds, Inc., St. Louis, MO) and water were available *ad libitum*.

F0 females were individually identified by eartag. F0 females were allowed to give birth and rear their litters. Litters were adjusted on pnd 4 to ten pups, maximizing the number of female pups. A total of 15 F1 females per group was assigned to each component in this study. F1 females were assigned to treatment groups by stratified randomization for body weight on pnd 21, so that mean body weight on pnd 21 did not differ among treatment groups. Selected female F1 weanlings were identified by eartag. They were housed in the study room(s) in polycarbonate solid-bottom cages with bedding and provided feed and water *ad libitum*. They were examined once daily by cage-side observation for morbidity or mortality while clinical observations or morbidity/mortality checks for the study animals.

All adult animals assigned to the study were euthanized by CO_2 asphyxiation. F1 pups culled on pnd 4 were sacrificed by decapitation.

Additional Methods. In order to allow for collection of individual feed consumption data, F1 weanling females were singly housed from pnd 21 to pnd 42/43. The feed was also analyzed by the manufacturer for the phytoestrogens daidzein, genistein, and glycitein. The singly housed animals, collection of feed consumption data, and the analysis of phytoestrogens in the feed were additions to the basic female pubertal study design. Additional information regarding these and other data collected during this study are presented in the Final Technical Study Report and its appendices.

Study Design

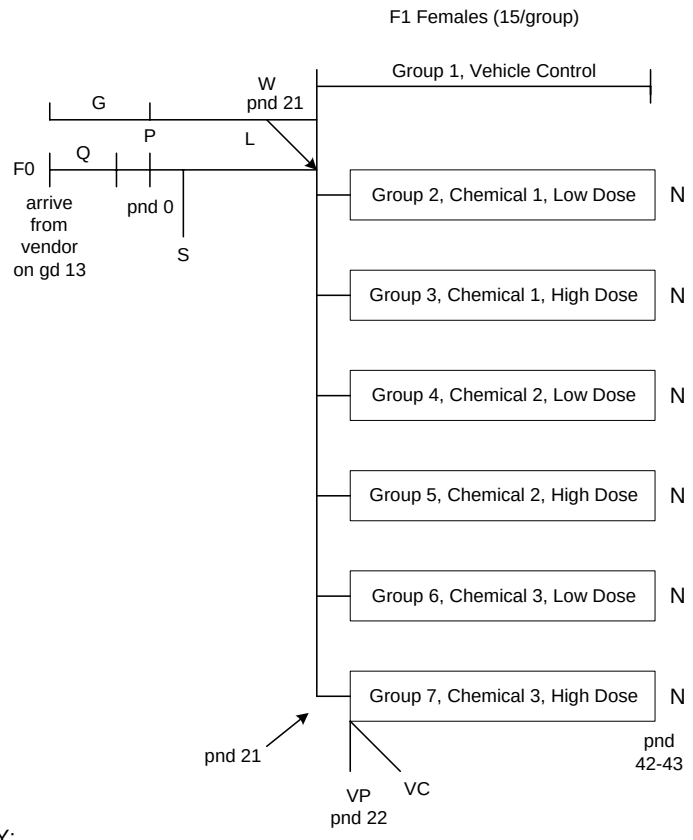
A graphic representation of the component study design is presented in Figure 1. The study began with 20 timed-mated F0 females in each component.

F0 Females

Beginning on gd 20, each female was examined twice daily (a.m. and p.m.) for evidence of littering. Females who were littering at morning and afternoon checks had this information recorded on the gestational sheet. Signs of dystocia or other signs of difficulty at parturition were also recorded, if observed. Any dams whose whole litters were born dead or died prior to pnd 21 were sacrificed, and the number of uterine implantation scars recorded. On pnd 21 of each F1 litter, each F0 dam was euthanized by CO_2 asphyxiation, and the carcass discarded.

Progeny (F1)

All pups were counted, sexed, weighed, and examined a soon as possible on the day of birth (designated as pnd 0) to determine the number of viable and stillborn members of each litter. Thereafter, litters were evaluated for survival, sex, gross observations, and body weights on pnd 4, 7, 14, and 21. Any pup that appeared moribund or died during lactation was necropsied, when possible, to investigate the cause of death and discarded. No organs were weighed or saved. On pnd 4, the size of each litter was adjusted to ten pups, maximizing the number of female pups retained. Natural litters with ten or fewer pups were not culled. All culled pups were sacrificed by decapitation. The F0 dams were allowed to rear their remaining F1 young to pnd 21. On pnd 21, each litter was weaned.



KEY:

——— No test chemical exposures to F0 dams or F1 offspring during gestation or lactation, no exposure of F1 females in Group 1; F1 females dosed with corn oil.

▭ Direct once daily gavage dosing with test chemical of F1 females starting on pnd 22 (see text)

Q = Quarantine (seven days, gd 13-20)

G = Gestation

P = Parturition (pnd 0)

L = Lactation

W = Wean (pnd 21) F1 pups; euthanize and discard F0 dams

S = Standardize litters to ten with maximum number of F1 female pups (discard culled pups)

VP = Acquisition of vaginal patency (evaluation began on pnd 22)

VC = Vaginal cytology (evaluation began on the day of VP)

N = Necropsy (see text)

Figure 1. General Component Study Design for the Female Pubertal Assay

F1 Females

When each F1 litter reached pnd 21, the F1 females from each pnd 21 (wean) date were weight ranked across litters (outliers, i.e., heaviest and lightest pups, were eliminated from selection). The selected females were eartagged and distributed across the seven groups of the component by stratified randomization (e.g., one of the seven heaviest selected females went into each of the seven treatment groups, etc.).

Beginning on pnd 22, each F1 female was dosed with a test material at one of the selected dose levels or the vehicle control (corn oil for all chemicals). EPA selected the seven test chemicals for this evaluation and selected the low and high target doses (in mg/kg/day) for each of them. Each animal was weighed every day prior to treatment and the body weight recorded. Treatments were administered daily by oral gavage from pnd 22 and continuing to pnd 42/43. The treatments were administered on a mg/kg body weight basis, adjusted based on the most recent body weight (Table 1).

Table 1. Study Design, Test Chemicals, and Target Doses

Group No.	No. F1 Females	Chemical	Dose (mg/kg/day)	Concentration (mg/ml)	Dose Volume (ml/kg)
		COMPONENT 1			
1	15	–[a]	0	0.0	5
2	15	Atrazine	75	15.0	5
3	15		150	30.0	5
4	15	Fenarimol	50	10.0	5
5	15		250	50.0	5
6	15	Methoxychlor	25	5.0	5
7	15		50	10.0	5
		COMPONENT 2			
8	15	Propylthiouracil	2	0.4	5
9	15		25	5.0	5
10	15	Bisphenol A	400	80.0	5
11	15		600	120.0	5
12	15	Ketoconazole	50	10.0	5
13	15		100	20.0	5
14	15	–[a]	0	0.0	5

[a] Corn oil, vehicle control

Clinical observations of F1 female study animals were documented at least once daily on pnd 21 (prior to dosing period) and at least twice daily, at dosing and one to two hours postdosing, throughout the dosing period (pnd 22 to pnd 42 or 43). All F1 females were weighed in the morning on pnd 21 and every morning during the dosing period on pnd 22 to pnd 42/43, for adjustment of dosing volume based on the most recent body weight. Daily body weights and body weight gains were reported and statistically analyzed.

Beginning on pnd 22, each F1 study female was examined daily for vaginal patency. The appearance of a small "pin hole," a vaginal thread, as well as complete vaginal opening was recorded on the days they were observed. The day of complete vaginal patency was the endpoint used in the analysis for the age of vaginal opening. Body weight at acquisition of complete vaginal patency was recorded.

Beginning on the day of vaginal opening and continuing until pnd 42/43, daily vaginal smears were obtained from each F1 female and evaluated to determine the age at first estrus, age at the first complete vaginal cycle, and/or any effects on estrous cyclicity. The number of animals in each treatment group that went through at least one complete cycle, and the number of days each animal spent in each stage of the estrous cycle was calculated. Analysis based both on pnd and by day-since-vaginal-patency was conducted for the onset of estrus. Indicators of estrous cyclicity were compared between control and treated groups.

Additional In-Life Data

Feed consumption for the individually-housed F1 weanling females was recorded daily and reported as g/day and as g/kg body weight/day. Calculation of these data were additions to the basic female pubertal study design. Additional information regarding these data and other data collected during this study are available in the Final Technical Study Report and its appendices.

Necropsy for pnd 42/43 F1 Females

Blood Collection and Hormone Assays

At scheduled necropsy of the F1 females, after terminal anesthesia (CO_2 asphyxiation), the females were weighed and the maximum amount of blood was taken by external cardiac puncture and placed in a labeled tube. The blood was allowed to clot and centrifuged under refrigeration. The resulting serum was analyzed for T4 and TSH. All assays were counted in a Packard Biosciences Cobra II Series Model 5002 gamma counter using RIASMART software. The rat thyroid-stimulating hormone (rTSH) RIA used was a no-extraction, double antibody ^{125}I RIA (Amersham Biosciences, Piscataway, NJ) which utilized rTSH antibody, ^{125}I-rTSH, rTSH calibrators as the standard curve, and a

precipitating solution consisting of donkey anti-rabbit serum coated onto magnetizable polymer particles. The sensitivity of this assay was 0.5 ng/tube. Results were reported as ng/ml.

The T4 RIA used was a no-extraction, solid-phase ^{125}I RIA which utilized T4-specific antibody-coated tubes and ^{125}I-T4 (DPC, Los Angeles, CA). The T4 (Sigma, St. Louis, MO) standard curve was prepared in RIA Buffer I (0.01 M sodium phosphate plus 0.85% [w/v] sodium chloride with 0.1% [w/v] sodium azide and 1% [w/v] bovine serum albumin, pH 7.6). T4 controls were prepared in the same matrix as unknown samples by adding known concentrations of T4 to female serum. The sensitivity of this assay was 0.25 µg/dL. Results were reported as µg/dL.

Gross Examination and Histopathology

Once each F1 female was bled, the animal was necropsied and internal thoracic and abdominal organs and cavities examined. Any abnormalities were documented. The following organs were dissected out and weighed: liver, kidneys (paired), adrenal glands (paired), pituitary, ovaries (paired), uterus (with and without fluid), and thyroid (with attached portions of trachea). For the uterine dissection, care was taken to remove mesenteric fat from the uterine horns and not damage the uterus so that the uterine fluid was retained. The uterus (without ovaries) was carefully dissected and trimmed of fascia and fat to avoid loss of luminal contents. The vagina was removed from the uterus at the level of the uterine cervix.

Tissues taken at necropsy were placed in fixative (10% neutral buffered formalin or Bouin's) and then transferred to Experimental Pathology Laboratories (EPL) for processing. In Component 1, the ovaries, uterus, and thyroid with attached portions of trachea were placed in Bouin's fixative for 24 hours, after which they were rinsed and stored in 70% alcohol until embedded in paraffin. The thyroid was weighed at EPL, after removal of the trachea. The tissues were sectioned at 3-5 microns and stained with hematoxylin and eosin (H and E) for subsequent histological evaluations. Pathologic evaluation of the thyroid revealed that fixation in Bouin's fixative rendered the tissue too dark and too brittle for optimal evaluation. The thyroids from animals in Component 2 were fixed in 10% neutral buffered formalin. Stained sections were evaluated for pathologic abnormalities and potential treatment-related effects by an AVCP board-certified veterinary pathologist.

Statistical Analyses

All data for a single chemical (two doses) and a concurrent vehicle control group were analyzed using either parametric ANOVA under the standard assumptions or robust regression methods (Zeger and Liang, 1986; Royall, 1986; Huber, 1967) which do not assume homogeneity of variance or normality. The homogeneity of variance assumption was examined via Levene's test (Levene, 1960). When Levene's test indicated lack of homogeneity of variance ($p<0.05$), robust regression methods were

used to test all treatment effects. The robust regression methods use variance estimators that make no assumptions regarding homogeneity of variance or normality of the data. They were used to test for linear trends across dose as well as overall treatment group differences (via Wald chi-square tests). Significant overall treatment effects were followed by single degree-of-freedom *t*-tests for exposed vs. control group comparisons, if the overall treatment effect was significant. When Levene's test did not reject the hypothesis of homogeneous variances, standard ANOVA techniques were applied for comparing the treatment groups. The GLM procedure in SAS® Version 8 (SAS Institute, Inc., 1999a,b,c,d,e; 2000) was used to test for linear trend, evaluate the overall effect of treatment and, when a significant treatment effect is present, to compare each exposed group to control via Dunnett's test (Dunnett, 1955, 1964). Standard ANOVA methods, as well as Levene's test, are available in the GLM procedure of SAS®, and the robust regression methods were available in the REGRESS procedure of SUDAAN® Release 7.5.4 (Shah et al., 1997) or Release 8.0 (RTI, 2001). Organ weights were also analyzed by Analysis of Covariance (ANCOVA) using the body weight at necropsy as the covariate. When statistically significant effects were observed, treatment means were examined further using LSMeans. The unit of comparison was the weanling F1 female offspring on study.

A test for statistical outliers was performed in the UNIVARIATE procedure of SAS® Version 8 (SAS Institute, Inc., 1999a,b,c,d,e; 2000) on F1 female body and organ weights. If examination of pertinent study data did not provide a plausible biologically sound reason for inclusion of the data flagged as "outlier," the data was excluded from summarization and analysis and was designated as outliers. For all statistical tests, $p \leq 0.05$ (one- or two-tailed) was used as the criterion for significance.

Personnel

This study was conducted at RTI International, Research Triangle Park, NC, under contract to Battelle, Columbus, OH. Dr. David P. Houchens, EDSP Program Manager, was the Sponsor's Representative. Dr. R.W. Tyl served as Project Toxicologist. Dr. J. D. George served as Study Director. Reproductive and Developmental Toxicology personnel included Ms. M.C. Marr (Laboratory Supervisor), Ms. C.B. Myers (Reproductive Toxicity Study Supervisor and Data Analyst), Mr. W.P. Ross, Ms. M.C. Rieth, Ms. V.I. Wilson, Ms. L.B Pelletier, Ms. M.P. Gower, Ms. N.M. Kuney, Ms. R.T. Krebs, Ms. S.W. Pearce, Ms. K.D. Vick, Ms. L. McDonald, Ms. A.J. Parham, Mr. M.D. Crews, Mr. C.G. Leach, Ms. A. B. Goodman, and Mr. T.W. Wiley. Bulk chemical analysis and handling, dose formulation, and dose formulation analysis were provided by the Sponsor through Dr. E.A. Crecelius, PNNL, Battelle Marine Sciences Laboratory, Sequim, WA. Mr. M.M. Veselica (Supervisor, RTI Materials Handling Facility), Mr. D.L. Hubbard, and Mr. R.A. Price provided receipt and disbursement of dose formulations at RTI. Animal care was provided by Dr. D.B. Feldman, DVM, ACLAM, RTI Veterinarian, and Mr. F.N. Ali, Manager of RTI Animal Research Facility. RTI Quality Assurance personnel were Ms. D.A. Drissel, Ms. D.J. Smith, Ms. M.D. Phillips, Ms. T.M. Kenney, Ms. C. Ingalls, and Ms. M. Oh. Ms. K.D. Andrews, QA Consultant, audited the hormone data and analysis.

The final report was prepared by Dr. J.D. George, with assistance from Dr. R.W. Tyl, Ms. B. Hamby, Ms. C.B. Myers, and Ms. M.C. Marr. Ms. C.B. Myers was responsible for data compilation and statistical analyses, and Mr. T.W. Wiley was responsible for data entry. Ms. M.C. Marr was responsible for all activities concerning organization and custody of the study records and for archiving the study records. Ms. D. Bynum and Ms. K. Kehagias provided secretarial assistance.

Compliance

All records, data, biological specimens, and reports will be maintained in storage for the period specified by the contract or for as long as the quality of the preparation affords evaluation, whichever is less. Quality control (QC) and quality assurance (QA) procedures followed those outlined in the Quality Assurance Project Plan (QAPP) prepared for this study and in accordance with the Quality Management Plan (QMP) for this project. The RTI Animal Research Facility is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), International. Animals were housed, handled, and used according to the NRC Guide (NRC, 1996).

RESULTS

Component 1

Control F1 Females

Fifteen untreated F1 females were assigned to the control group for Component 1. These animals served as the concurrent control group for the animals in Component 1 that were treated with atrazine, fenarimol, and methoxychlor.

In-Life Data From F1 Females Treated With Atrazine

Fifteen untreated F1 females were assigned to the 0, 75, or 150 mg/kg/day atrazine group. No animals assigned to the control group or either atrazine-treated group died prior to scheduled necropsy. Body weights at weaning (pnd 21) and on the day of initiation of dosing (pnd 22) were equivalent across treatment groups (Figure 2 and Table 2-A). Daily body weights for F1 females were significantly decreased in the high-dose group compared to the control group from pnd 23 to scheduled necropsy on pnd 42 or 43. In the low dose group, body weight was decreased compared to the control group on pnd 31, 33, 34, 35, 37, 38, 39, 40, 41, and 42, but not prior to pnd 31, or on pnd 32, 36, or 43. Body weight change was significantly less in both atrazine-treated groups compared to the control group for pnd 22 to 42 (Table 2-A). On pnd 42, body weight was significantly reduced at both doses of atrazine, with the low and high dose animals weighing 93.3% and 87.7%, respectively, of the control animal weight (Table 2-A).

Clinical observations were noted in the atrazine-treated groups and included rooting postdosing, salivation prior to dosing, and sore(s) in 4, 9, and 1 animal(s) in the high-dose group, respectively, and the same observations in 1, 6, and 1 animal(s) in the low-dose group, respectively.

Treatment with atrazine resulted in a dose-related delay in vaginal opening, which was significant at the high dose (36.4 days vs. 32.9 days for the control group; Table 2-A). There was no significant effect of treatment on the average body weight on the day of acquisition of vaginal opening, although body weights tended to increase with dose, due to the delay in the acquisition of this landmark. It is noteworthy that due to the delay in vaginal opening and subsequent first estrus, four of the high-dose animals could not be evaluated for estrus cyclicity, since there were not enough observation days after first estrus and prior to scheduled necropsy to collect an adequate number of smears. In addition, one female in the high-dose group was acyclic, although she exhibited vaginal opening and first estrus. It is not known whether this female would have exhibited cycling if the observation period had been extended beyond pnd 42/43.

Necropsy and Histopathological Data from F1 Females Treated with Atrazine

At necropsy, average body weight exhibited a dose-related decreasing trend, with the low and high dose atrazine-treated groups significantly below the control group (Table 3-A). Absolute pituitary weight was decreased at the high dose, as was paired ovary weight, whereas liver weight exhibited a dose-related decreasing trend without significant pairwise comparisons. When organ weights were adjusted with respect to necropsy body weight, adjusted liver weight increased in a dose-related manner that was significant at both doses of atrazine. In addition, adjusted paired kidney weight exhibited an increasing trend, with the high-dose value significantly greater than the control group value. Atrazine treatment had no significant effect on thyroid, adrenal, or uterine weights (with or without fluid), or on T4 or TSH levels. Atrazine at 75 or 150 mg/kg/day did not alter the incidence of females in diestrus, proestrus/estrus, or not cycling at necropsy (Table 3-A).

Gross necropsy findings were minimal, and included one animal each in the control- and high-dose group with hydronephrosis, one animal in the high-dose group with a uterine cyst, and one animal each in the control and low-dose group with fluid in the uterus. No treatment-related histopathology was observed.

In-Life Data from F1 Females Treated with Fenarimol

Fifteen untreated F1 females were assigned to each of the 0, 50, or 250 mg/kg/day fenarimol groups. No animals assigned to the control group or either fenarimol-treated group died prior to scheduled necropsy. Body weight at weaning (pnd 21) and on the day of initiation of dosing (pnd 22) was equivalent across treatment groups (Figure 3 and Table 2-B). Daily body weights for F1 females were significantly decreased in the high-dose group compared to the control group from pnd 23 to scheduled necropsy on pnd 42, but not on pnd 43. No significant effect on daily body weight was observed for the low-dose group (Figure 3). Body weight change was significantly less in the high dose fenarimol-treated group compared to the control group for pnd 22 to 42, but was not affected by treatment at the low dose (Table 2-B). On pnd 42, body weight was significantly depressed at the high dose; the low and high dose animals weighed 102 and 90.8%, respectively, of the control value (Table 2-B).

Clinical observations were noted in the fenarimol-treated groups and included rooting postdosing, salivation prior to dosing, and sore(s) in five, seven, and two animal(s) in the high-dose group, respectively, and rooting postdosing and salivation prior to dosing in six and four animal(s) in the low-dose group, respectively.

Treatment with fenarimol did not alter the average postnatal day of vaginal opening (Table 2-B). Average body weight on the day of acquisition was significantly decreased at the high dose. Two of the

high-dose animals could not be evaluated for estrus cyclicity, since there were not enough days after first estrus prior to scheduled necropsy to collect an adequate number of smears. In addition, two females in the low-dose group were acyclic, although they exhibited vaginal opening and first estrus. It is not known whether these females would have exhibited cycling if the observation period had been extended beyond pnd 42/43.

Necropsy and Histopathological Data from F1 Females Treated with Fenarimol

At necropsy, average body weight was significantly decreased at the high dose of fenarimol compared to the control group (Table 3-B). Absolute pituitary weight was decreased at the high dose, as was the uterine weight (without fluid), whereas liver weight exhibited a dose-related increasing trend with an increase of the high dose. Paired kidney and ovary weights exhibited a decreasing trend. When organ weights were adjusted with respect to necropsy body weight, pituitary weight was still significantly decreased compared to the control group, whereas liver weight increased in a dose-related manner that was significant at both doses of fenarimol. In addition, adjusted paired ovary weight and uterine weight without fluid exhibited a decreasing trend. Fenarimol treatment had no significant effect on thyroid or adrenal weights. T4 levels (microg/dL) were significantly decreased at the high dose (by 16% compared to the control value), whereas TSH levels (ng/ml) exhibited a dose-related increase that was significant at both doses of fenarimol. The low- and high-dose fenarimol-treated groups exhibited a 30.2% and 104.3% increase, respectively, in the level of TSH compared to the control group value. Fenarimol did not appear to have an effect on the number of animals in diestrus, proestrus/estrus, or not cycling at necropsy (Table 3-B).

Gross necropsy findings were minimal and included one animal in each group with hydronephrosis, one animal in the high dose group with a shortened right uterine horn, one animal in the low-dose group with a uterine cyst, and one to three animals in the control- and high-dose group with a fluid filled uterus. Minimal follicular hypertrophy of the thyroid was observed in 5/15 of the high dose females.

In-Life Data from F1 Females Treated with Methoxychlor

Fifteen untreated F1 females were assigned to the 0, 25, or 50 mg/kg/day methoxychlor groups. One animal in the 25 mg/kg/day methoxychlor-treated group was found dead on pnd 38 prior to scheduled necropsy. Thus, 15, 14, and 15 animals in the control, low, and high dose groups were available for full evaluation in this study. Body weight at weaning (pnd 21) and on the day of initiation of dosing (pnd 22) was equivalent across treatment groups (Table 2-C and Figure 4). In addition, daily body weights for F1 females were unaffected by methoxychlor treatment through pnd 34. From pnd 35 through necropsy on pnd 42 or 43, body weight exhibited a decreasing trend, with a significant effect at the high dose for all time points from pnd 36 to 42. No significant effect on daily body weight was

observed at the low dose of methoxychlor (Figure 4). However, body weight change was significantly less in both methoxychlor-treated groups compared to the control group for pnd 22 to 42 (Table 2-C). On pnd 42, body weight was significantly decreased at the high dose. The low and high dose animals weighed 95.1 and 93.6% of the control animal weight on pnd 42, respectively (Table 2-C).

Clinical observations were noted in the methoxychlor-treated groups and consisted of rooting, postdosing, in one animal in the high-dose group and salivation prior to dosing in one and three animal(s) in the low and high-dose groups, respectively. As noted previously, one animal was found dead during the postwean treatment period..

Treatment with methoxychlor resulted in a dose-related acceleration in vaginal opening that was significant in both treated groups (Table 2-C). The average postnatal day of vaginal opening was 29.8 and 27.4 for the low- and high-dose groups, respectively, compared to 32.9 days for the control group. Body weight on the day of acquisition of vaginal opening was significantly depressed for both the low and the high dose methoxychlor-treated groups, secondary to the younger age of these animals at acquisition. All but one female in the low-dose group were observed to cycle.

Necropsy and Histopathological Data from F1 Females Treated with Methoxychlor

At necropsy, average body weight exhibited a dose-related decreasing trend, with the high-dose methoxychlor-treated group significantly below the control group (Table 3-C). Absolute paired ovary weight exhibited a decreasing trend. When organ weights were adjusted with respect to necropsy body weight, adjusted paired ovary weight still exhibited a decreasing trend, and was significantly reduced at the high dose. Methoxychlor treatment had no significant effect on pituitary, thyroid, liver, adrenal, kidney, or uterine weights (with or without fluid), or on T4 or TSH levels (Table 3-C).

Gross necropsy findings were minimal, and included one animal in each group with hydronephrosis, and one to six animals in each treatment group with uterine fluid. No treatment-related histopathology was observed. Methoxychlor did not appear to have an effect on the number of animals in diestrus, proestrus/estrus, or not cycling at necropsy.

Component 2

Control F1 Females

Fifteen untreated F1 females were assigned to the control group for Component 2. These animals served as the concurrent control group for the animals in Component 2 that were treated with bisphenol A, ketoconazole, and propylthiouracil.

In-Life Data from F1 Females Treated with Bisphenol A

Fifteen untreated F1 females were assigned to the 0, 400, or 600 mg/kg/day bisphenol A groups. One animal in the control and high-dose groups, and 4 animals in the low-dose group were either found dead or euthanized due to moribundity prior to scheduled necropsy. Thus, 14, 11, and 14 females in the control, low, and high-dose groups were available for full evaluation in this study. Body weight at weaning (pnd 21) and on the day of initiation of dosing (pnd 22) was equivalent across treatment groups (Figure 5 and Table 2-D). Daily body weights for F1 females were not significantly affected by treatment from pnd 23 to 25. Thereafter, body weight was significantly decreased in the high-dose group compared to the control group from pnd 26 to scheduled necropsy on pnd 42 or 43. In the low-dose group, body weight was decreased compared to the control group on pnd 29 and from pnd 31 to scheduled necropsy. Body weight change was significantly decreased in both bisphenol A-treated groups compared to the control group for pnd 22 to 42 (Table 2-D). Body weight on pnd 42 was significantly depressed in both bisphenol A-treated groups, with the low and high dose animals weighing 88.7 and 85.8% of the control weight on pnd 42, respectively (Table 2-D).

Clinical observations were noted in the bisphenol A-treated groups and included efflux of the dosing solution, gasping, rooting postdosing, and salivation prior to dosing in 1, 1, 7, and 12 animals in the low-dose group, respectively, efflux of dosing solution, piloerection, audible respiration, rooting postdosing, and salivation prior to dosing in 2, 1, 2, 15, and 10 animals in the high-dose group, respectively.

Treatment with bisphenol A did not affect the average day of vaginal opening (32.3–33.0 days; Table 2-D). The average body weight on the day of acquisition of vaginal opening exhibited a decreasing trend, and animals in the high-dose group weighed significantly less than the control group. One female in the low-dose group was acyclic, although she exhibited vaginal opening and first estrus. Three females in the high-dose group were acyclic. It is not known whether these females would have ultimately exhibited cycling if the observation period had been extended beyond pnd 42/43.

Necropsy and Histopathological Data from F1 Females Treated with Bisphenol A

At necropsy, average body weight exhibited a dose-related decreasing trend, with the low and high-dose bisphenol A-treated groups significantly below the control group (Table 3-D). In addition, absolute liver, paired kidney, and paired ovary weights decreased in a dose-related manner, with both treated groups significantly below the control group. Absolute uterine weight (with or without fluid) exhibited a dose-related decreasing trend; the high-dose group was significantly depressed for uterine weight without fluid. There was no effect of bisphenol A treatment on absolute pituitary or thyroid gland weights. When organ weights were adjusted with respect to necropsy body weight, adjusted paired ovary weight exhibited a decreasing trend, but there was no other significant effect. Bisphenol A

treatment had no significant effect on T4 or TSH levels. Bisphenol A did not appear to have an effect on the number of animals in diestrus, proestrus/estrus, or not cycling at necropsy (Table 3-D).

Gross necropsy findings were minimal and included three animals in the control and high-dose groups with hydronephrosis. Ovarian hypoplasia was observed in 3/14 animals at the high dose. These ovaries exhibited a complete absence of corpora lutea and an apparent reduction or absence of Graffian follicles. As noted above, absolute and adjusted (pnd 21) paired ovary weights were significantly decreased at the high dose, whereas adjusted (necropsy) paired ovarian weight exhibited a dose-related decreasing trend, only.

In-Life Data from F1 Females Treated with Ketoconazole

Fifteen untreated F1 females were assigned to the 0, 50, or 100 mg/kg/day ketoconazole group. One animal in the control group died prior to scheduled necropsy. Thus, 14, 15, and 15 F1 females were available for evaluation in this study. Body weights at weaning (pnd 21), on the day of initiation of dosing (pnd 22), and on pnd 23 were equivalent across treatment groups (Figure 6 and Table 2-E). Daily body weights for F1 females exhibited a decreasing trend from pnd 24 to scheduled necropsy on pnd 42. The high-dose group was significantly below the control group on pnd 25 through 35 and pnd 37 to pnd 42. Body weight change was significantly less in the high dose ketoconazole-treated group compared to the control group for pnd 22 to 42 (Table 2-E). On pnd 42, the high dose animals weighed less than the controls. Animals in the low and high dose groups weighed 100.2 and 92.1% of the control value on pnd 42 (Table 2-E).

Clinical observations noted in the ketoconazole-treated groups included efflux of dosing solution, rooting postdosing, and salivation prior to dosing in one, five, and six animal(s) in the high-dose group, respectively, and piloerection, rooting postdosing, and salivation prior to dosing in one, three, and six animal(s) in the low-dose group, respectively.

Treatment with ketoconazole had no significant effect on the average postnatal day of vaginal opening (Table 2-E). Body weight at acquisition was not affected by treatment. One animal in the control group, two animals in the low-dose group, and four animals in the high-dose group did not cycle.

Necropsy and Histopathological Data from F1 Females Treated with Ketoconazole

At necropsy, average body weight exhibited a dose-related decreasing trend, with the high-dose ketoconazole-treated group value significantly below the control group value (Table 3-E). Absolute paired ovary weight and uterine weight (with or without fluid) exhibited a dose-related decreasing trend, with the high-dose value significantly below the control group value. Absolute paired kidney weight exhibited an increasing trend. Absolute paired adrenal gland weight exhibited an increasing trend and

was also significantly increased at both doses of ketoconazole. When organ weights were adjusted with respect to necropsy body weight, liver weight, paired adrenal weight, and paired kidney weights increased in a dose-related manner that was significant at both doses of ketoconazole. Uterine weights without fluid exhibited a decreasing trend. Ketoconazole treatment had no significant effect on pituitary or thyroid gland weights, or on T4 or TSH levels. Ketoconazole did not appear to have an effect on the number of animals in diestrus, proestrus/estrus, or not cycling at necropsy (Table 3-E).

Gross necropsy finding were minimal and included eight animals in the low-dose group and five animals in the high-dose group with pale and/or enlarged adrenal glands, three animals in the control group and two animals in the low-dose group with hydronephrosis, and one animal in the low-dose group with a missing ovary. Cytoplasmic vacuolization of the corpora lutea was observed in 9/15 (60%) of the females exposed to the high dose of ketoconazole. These ovaries exhibited vacuoles within the cytoplasm of the corpora lutea cells. The severity was judged by a comparison of the average number and size of the vacuolated cells expressed as a percentage of the area of the corpora lutea affected by vacuolization, i.e., minimal = 6-25%, mild = 26 to 50%, moderate = 51-75%, and marked = 76-100%. Based on these criteria, the nine animals were scored as 1 minimal, 4 mild, 3 moderate, and 1 marked. In addition, 5/15 (33%) of the high-dose females exhibited mild ovarian hypoplasia (no corpora lutea present). Thus, these five animals could not be evaluated for corpora luteal cytoplasmic vacuolization. A total of 14/15 animals (93.3%) exhibited ovarian pathology. Subsequent evaluation of the low-dose group revealed that 12/15 females exhibited cytoplasmic vacuolization of the corpora lutea: 6 minimal, 4 mild, and 2 moderate. No animals exhibited ovarian hypoplasia. The absolute paired ovarian weight was significantly decreased at the high but not the low dose of ketoconazole. This change in organ weight likely reflects the ovarian hypoplasia observed in the high-dose animals.

In-Life Data from F1 Females Treated with Propylthiouracil

Fifteen untreated F1 females were assigned to the 0, 2, or 25 mg/kg/day propylthiouracil group. One animal assigned to the control group was found dead prior to scheduled necropsy. Thus, there were 14, 15, and 15 F1 females available for evaluation in the control, low- and high-dose groups, respectively. Body weights were equivalent across treatment groups from pnd 21 to pnd 31 (Figure 7 and Table 2-F). Thereafter, the high-dose group weighed significantly less than the control group. However, body weight change was significantly less at the high dose of propylthiouracil compared to the control group for pnd 22 to 42 (Table 2-F). On pnd 42, body weight was significantly less at the high dose compared to the control value (Figure 7 and Table 2-F). The low and high dose animals weighed 97.3 and 72.4% of the control animal weight on pnd 42 (Table 2-F).

Clinical observations were noted in the propylthiouracil-treated groups and included rooting postdosing in three animals at the low dose, and piloerection, rooting postdosing, and salivation prior to dosing in one, five, and six animal(s) in the high-dose group, respectively.

Treatment with propylthiouracil had no significant effect on the average postnatal day of vaginal opening (Table 2-F). Body weight at acquisition of vaginal opening exhibited a significant decreasing trend and treatment effect, but no pairwise differences between the treated and control groups (Table 2-F).

Necropsy and Histopathological Data from F1 Females Treated with Propylthiouracil

At necropsy, average body weight exhibited a dose-related decreasing trend, with the high-dose propylthiouracil-treated group significantly below the control group (Table 3-F). Absolute pituitary weight and uterine weight (with or without fluid) were unaffected by treatment. However, absolute weights for liver, paired adrenals, paired kidneys, and paired ovaries were decreased at the high dose, whereas absolute thyroid weight was increased at both doses of propylthiouracil. When organ weights were adjusted with respect to necropsy body weight, thyroid weight exhibited a dose-related increase, with values from both propylthiouracil-treated groups significantly greater than the control group value (4.3- and 9.5-fold for the low and high dose, respectively). No other differences were noted for other organ weights. As expected, T4 levels decreased in a dose-related manner with both propylthiouracil-treated groups significantly decreased compared to control values (59.0% and 86.3%, respectively). TSH levels were increased 5.4-fold and 12.6-fold in the low and high-dose propylthiouracil-treated groups, respectively, compared to the control group value. Propylthiouracil did not appear to have an effect on the number of animals in diestrus, proestrus/estrus, or not cycling at necropsy. (Table 3-F).

Gross necropsy findings were minimal, and included three, three, and one animals in the control-, low-, and high-dose groups with hydronephrosis, and one animal each in the high-dose group with a misshapen kidney, enlarged or enlarged/reddened thyroid gland, or fluid-filled uterus. All animals in the high-dose group exhibited thyroid follicular cell hypertrophy/hyperplasia, characterized by increased size and apparent number of follicular cells, reduction of follicular lumen size, and reduction in or absence of follicular colloid. The severity of these changes were scored as: minimal = multifocal follicles affected, with size and number of follicular cells slightly enlarged and increased; mild = diffuse change with further increased cell size and hyperplasia; moderate = enhanced severity with the presence of notable numbers of follicular cell mitoses; and marked = increased mitotic rate, some degenerative cells within the follicular epithelium, and obvious enlargement of the thyroid shape and size. Based on these criteria, no animals in the high-dose group scored minimal, two animals scored mild, nine moderate, and four marked. Based on these results, the low-dose thyroids were also examined, revealing that all 15 animals at the low dose also exhibited thyroid hypertrophy/hyperplasia. As expected, the severity was somewhat less, with scores of 1 minimal, 13 mild, 1 moderate, and no markedly affected animals. These changes were reflected in the dose-related increases in adjusted thyroid weight at both dose levels.

DISCUSSION AND CONCLUSION

This study was designed to gather information describing the conduct and usefulness of the female pubertal assay, that has been designated as a required Endocrine Disruptor Tier I screening protocol (EDSTAC, 1998), using compounds selected to aid in the optimization of the protocol. Currently in the prevalidation stage, the female pubertal assay provides a means of screening apical effects of endocrine disruptors that may alter a number of endocrine-dependent mechanisms, including estrogenic-, androgenic-, and thyroid hormone-related processes (Goldman et al., 2000). As summarized in Goldman et al. (2000), the female pubertal protocol should be able to detect alterations in sexual maturation and thyroid function. The endpoints in the current version of this protocol were chosen to reflect specific changes in pubertal development, thyroid function, or general toxicity. In an effort to evaluate the ability of this protocol to detect alterations each of these areas, the following compounds were tested: atrazine (affects the hypothalamus-pituitary axis in female rats and ovulation); fenarimol (a weak aromatase inhibitor); methoxychlor (a xeno-estrogen through α-estrogen receptor, anti-estrogen through β-estrogen receptor, and an anti-androgen through androgen receptor-mediated mechanism); bisphenol A (a weak environmental estrogen which binds to both the α and β estrogen receptor); ketoconazole (inhibits steroidogenesis in both sexes); and propylthiouracil (affects the thyroid directly, causing hypothyroidism). With respect to the test compounds, the results of this study are discussed below on how the protocol performed.

Atrazine. Atrazine, a chlorotriazine herbicide, has been shown to affect the hypothalamus-pituitary axis in female rats, delaying the onset of puberty (see Laws et al., 2000). In the present study, treatment with atrazine at 150 mg/kg/day delayed puberty, as evidenced by delayed vaginal opening. The postnatal day of vaginal opening exhibited a significant delay at the high dose (36.4 days vs. 32.9 days for the control group). Body weight and body weight gain were decreased at both dose levels during treatment, although not at acquisition of vaginal opening. Absolute, but not adjusted paired ovary weight was significant decreased at the high dose. There was no significant effect of treatment on uterine weight (absolute or adjusted for necropsy weight), circulating T4 or TSH levels, or histopathological changes in the thyroid, ovaries, or uterus.

Fenarimol. Fenarimol at 50 and 250 mg/kg/day did not alter the day of acquisition of vaginal opening compared to the control group, although body weight at acquisition was decreased at the high dose. In addition, body weight and body weight change were decreased at the high dose during the treatment period and at sacrifice. There was no effect of treatment on absolute or adjusted thyroid weight. Circulating T4 levels were significantly reduced at the high dose, whereas an increase in circulating TSH levels was observed at 50 and 250 mg/kg/day. These effects on thyroid hormone levels were consistent with the observation of minimal follicular hypertrophy in 33% of the high-dose thyroids. Absolute uterine weight (without fluid) was significantly reduced at the high dose, but exhibited no treatment effect when adjusted for necropsy weight and no treatment-related histopathology. Absolute

and adjusted paired ovary weight exhibited a decreasing trend, but the tissues exhibited no adverse histopathological effects.

Methoxychlor. Methoxychlor at 50 mg/kg/day decreased body weight in the high dose during the last 6 days of dosing. In addition, body weight change for the treatment period was decreased at both the 25 and 50 mg/kg/day dose level. Accelerated vaginal opening was observed at both 25 and 50 mg/kg/day methoxychlor, in the presence of decreased body weight on the day of acquisition. Decreased adjusted paired ovary weight was observed at the high dose. There was no treatment effect on absolute or any other adjusted organ weights, thyroid hormone levels, or histopathology.

Bisphenol A. Treatment with bisphenol A resulted in decreased body weight gain (pnd 22 to 42) and body weight at necropsy (pnd 42). The postnatal day of vaginal opening was not affected by bisphenol A treatment. Body weight of the high-dose animals was significantly reduced on the day of acquisition, compared to the controls. Absolute, but not adjusted uterus without fluid weight was decreased at the high dose. Absolute paired ovary weight was decreased at both doses, whereas adjusted paired ovary weight exhibited a decreasing trend. Ovarian hypoplasia was observed in 21% of the high dose animals. Circulating thyroid hormone levels were unaffected by treatment.

Ketoconazole. The day of vaginal opening was not affected by ketoconazole treatment. Effects at the high dose included a decrease in absolute paired ovary and uterus (with or without fluid) weight. Adjusted liver and paired kidney weight was increased at both doses of ketoconazole. Ovarian pathology increased in incidence and severity with dose. Cytoplasmic vacuolization of the corpora lutea was observed in 12/15 and 9/15 of the low and high-dose animals, respectively. The five remaining animals in the high-dose group exhibited a complete absence of corpora lutea. Ketoconazole treatment did not adversely affect circulating thyroid hormone levels.

Propylthiouracil. As expected, propylthiouracil produced a decrease in T4 and an increase in TSH circulating levels, increased adjusted thyroid weight, and thyroid follicular cell hypertrophy/hyperplasia at both 2 and 25 mg/kg/day. Acquisition of vaginal opening was not affected by propylthiouracil treatment. In addition, other than the thyroid, treatment with propylthiouracil did not affect adjusted organ weights.

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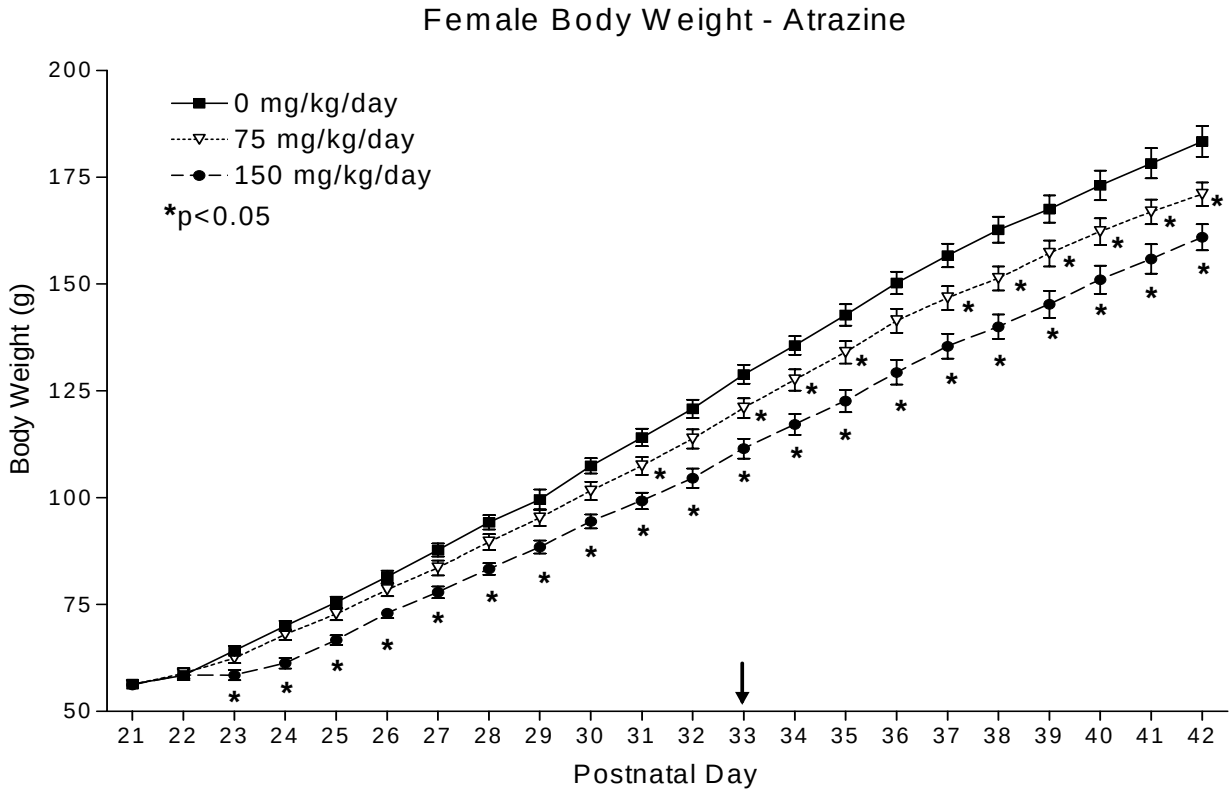

Figure 2. Mean daily body weight in pubertal females treated with 0, 75, or 150 mg/kg/day Atrazine. The mean day of acquisition of vaginal opening for the control group is indicated by the arrow. Data are presented as mean ± S.E.M. (n = 15 animals per group). * = p<0.05, Dunnett's Test.

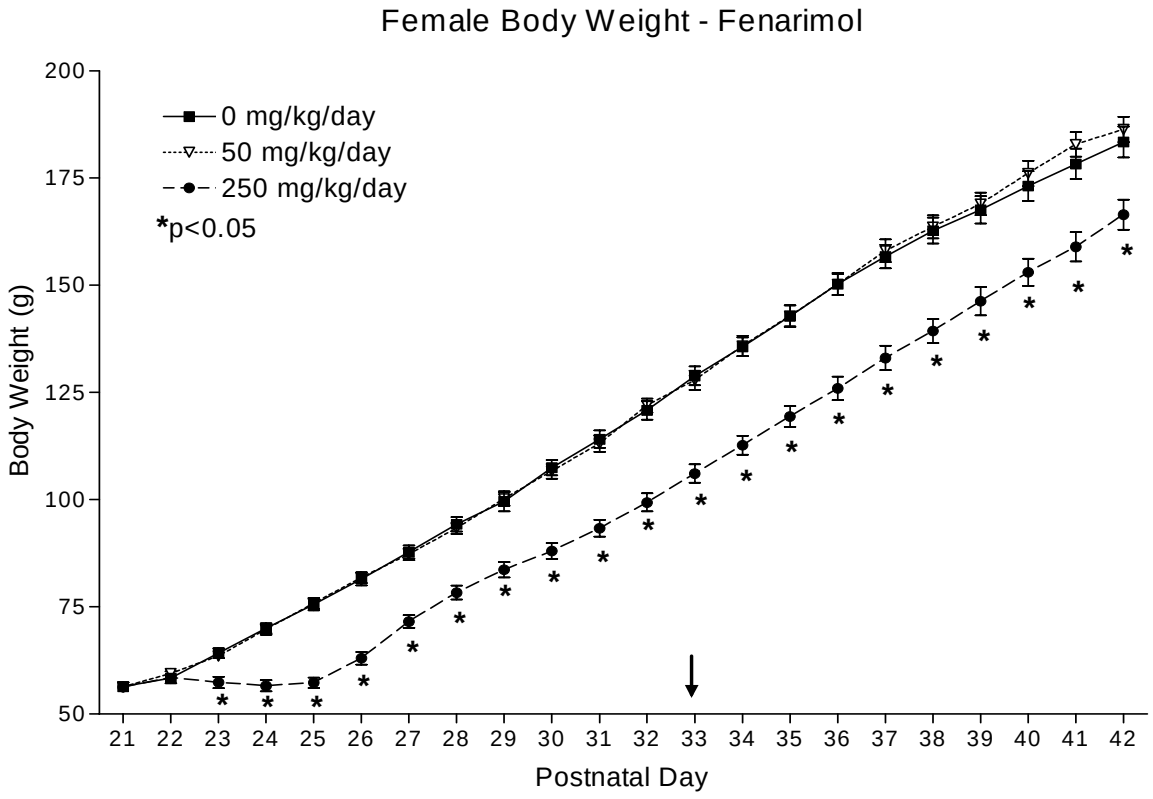

Figure 3. Mean daily body weight in pubertal females treated with 0, 50, or 250 mg/kg/day Fenarimol. The mean day of acquisition of vaginal opening for the control group is indicated by the arrow. Data are presented as mean ± S.E.M. (n = 15 animals per group). * = p<0.05, Dunnett's Test.

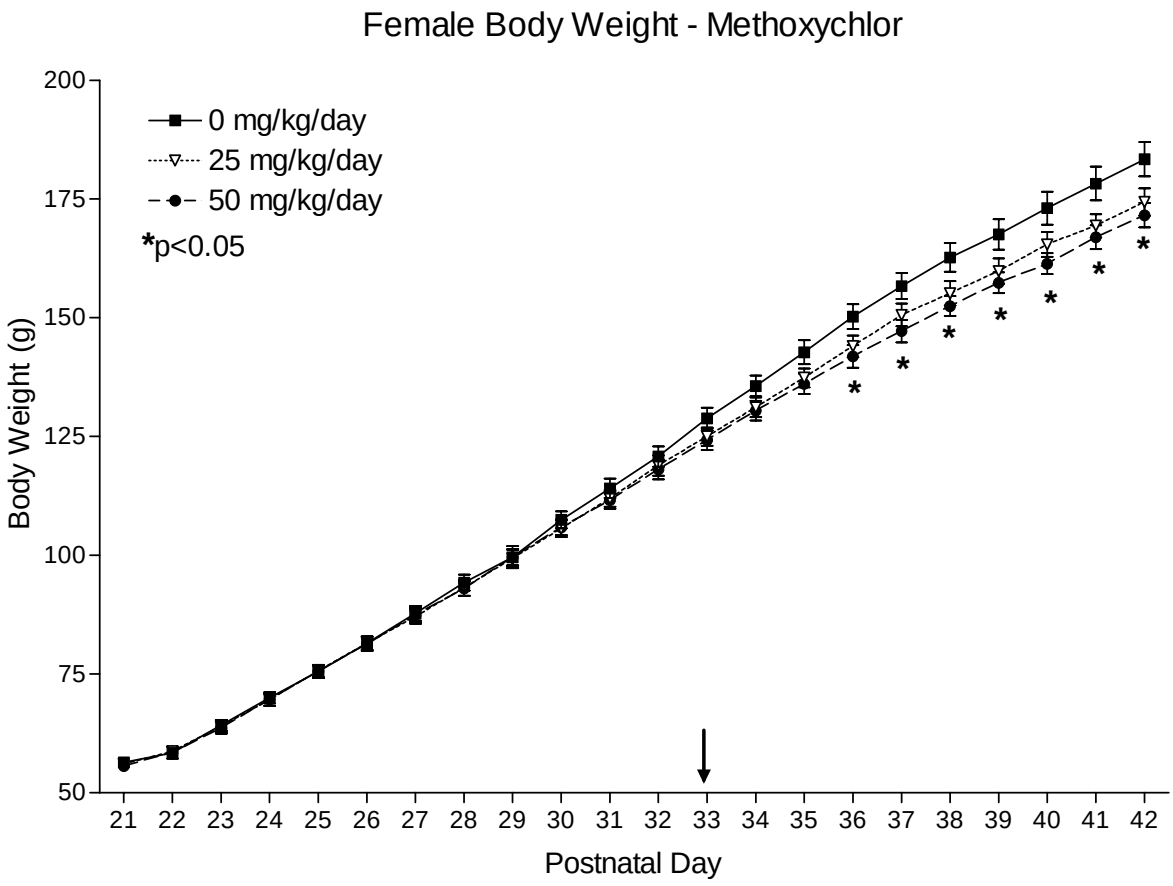

Figure 4. **Mean daily body weight in pubertal females treated with 0, 25, or 50 mg/kg/day Methoxychlor. The mean day of acquisition of vaginal opening for the control group is indicated by the arrow. Data are presented as mean ± S.E.M. (n = 14-15 animals per group). * = p<0.05, Dunnett's Test.**

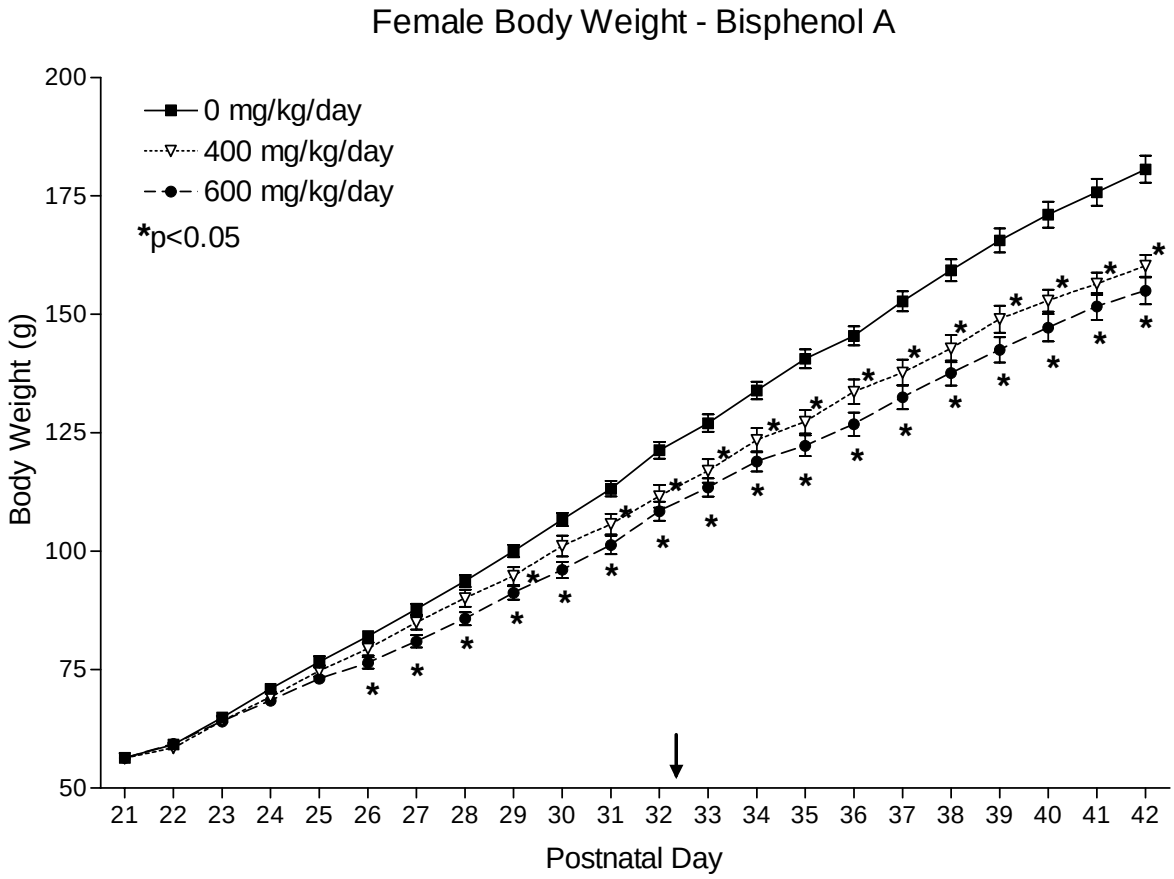

Figure 5. Mean daily body weight in pubertal females treated with 0, 400, or 600 mg/kg/day Bisphenol A. The mean day of acquisition of vaginal opening for the control group is indicated by the arrow. Data are presented as mean ± S.E.M. (n = 11-15 animals per group). * = p<0.05, Dunnett's Test.

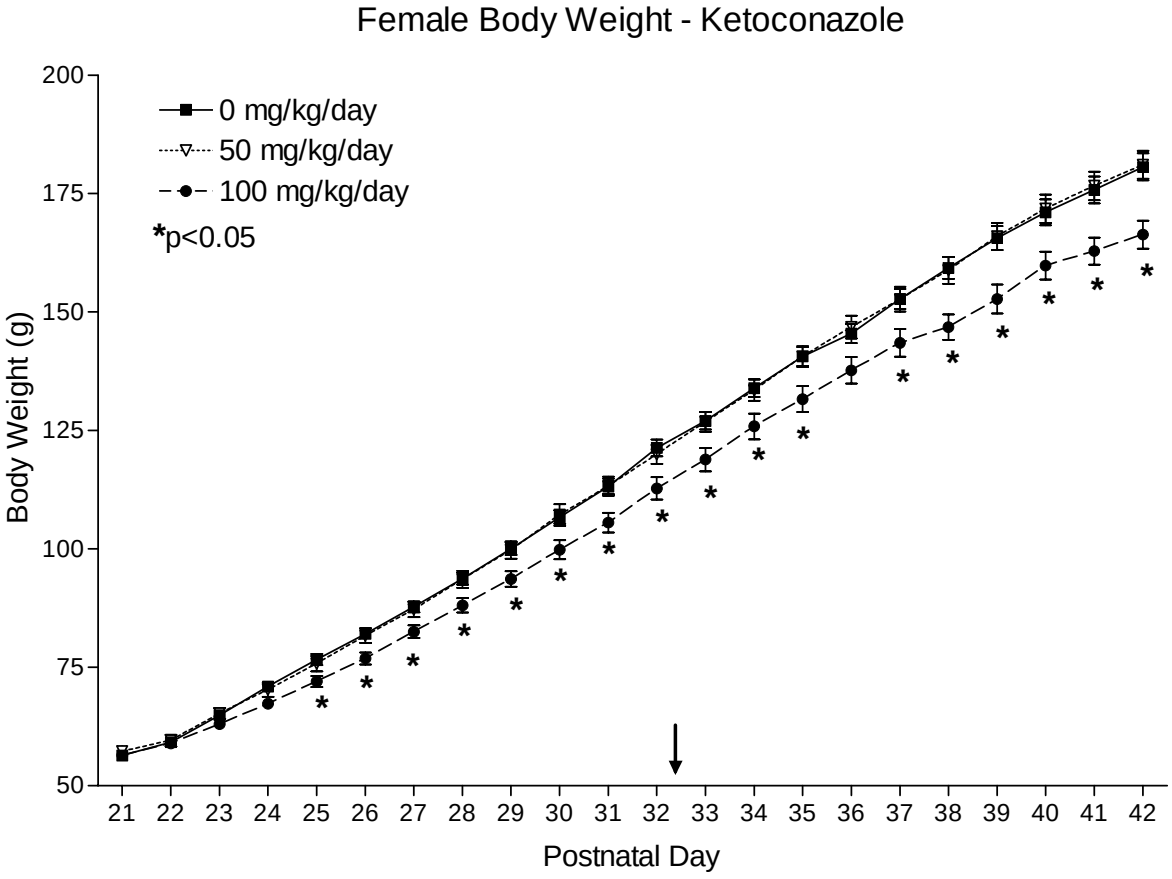

Figure 6. Mean daily body weight in pubertal females treated with 0, 50, or 100 mg/kg/day Ketoconazole. The mean day of acquisition of vaginal opening for the control group is indicated by the arrow. Data are presented as mean ± S.E.M. (n = 14-15 animals per group). * = p<0.05, Dunnett's Test.

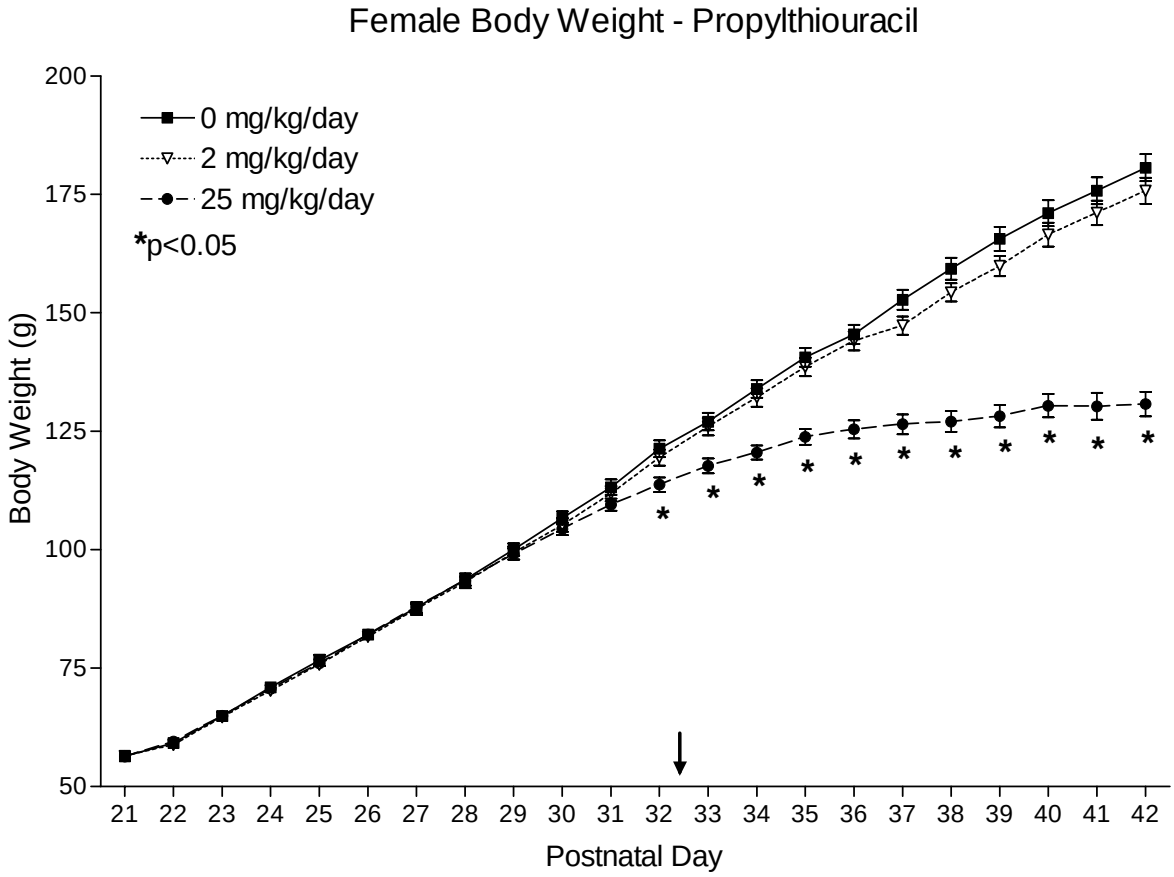

Figure 7. Mean daily body weight in pubertal females treated with 0, 2, or 25 mg/kg/day Propylthiouracil. The mean day of acquisition of vaginal opening for the control group is indicated by the arrow. Data are presented as mean ± S.E.M. (n = 14-15 animals per group). * = p<0.05, Dunnett's Test.

Table 2-A. Vaginal Opening and Body Weight in Atrazine-Treated F1 Females[a]

Parameter	Atrazine (mg/kg/day, po)		
	0	75	150
Age at VO (days)	32.9 [b, c]	34.2	36.4 *
	± 0.4	± 0.4	± 0.6
BW at VO (grams)	127.89	128.07	129.81
	± 3.20	± 3.03	± 2.91
Initial BW (pnd 22, grams)	58.42	58.91	58.55
	± 0.97	± 1.16	± 1.22
Final BW (pnd 42, grams)	183.39 [b, c]	171.02 *	160.98 *
	± 3.61	± 2.73	± 3.06
Final BW as % of control (pnd 42)		93.3	87.7
BW gain (pnd 22 to 42, grams)	124.97 [b, c]	112.12 *	102.43 *
	± 3.00	± 2.45	± 2.29

[a] Mean ± SE (n = 15 for each treatment group).

[b] Significant Test for Linear Trend ($p<0.05$).

[c] Significant treatment effect by ANOVA ($p<0.05$).

*Significantly different from control by Dunnett's Test ($p<0.05$).

Table 2-B. Vaginal Opening and Body Weight in Feniramol-Treated F1 Females [a]

Parameter	Feniramol (mg/kg/day, po)		
	0	50	250
Age at VO (days)	32.9[d]	33.7	34.2
	± 0.4	± 0.4	± 0.7
BW at VO (grams)	127.89 [b, c, d]	132.62	114.53 *
	± 3.20	± 2.45	± 4.35
Initial BW (pnd 22, grams)	58.42	59.44	58.56
	± 0.97	± 1.06	± 1.43
Final BW (pnd 42, grams)	183.39 [b, c]	186.32	166.48 *
	± 3.61	± 2.93	± 3.51
Final BW as % of control (pnd 42)		102	90.78
BW gain (pnd 22 to 42, grams)	124.97 [b, c]	126.88	107.92 *
	± 3.00	± 2.77	± 2.81

[a] Mean ± SE (n = 15 for each treatment group).

[b] Significant Test for Linear Trend ($p<0.05$).

[c] Significant treatment effect by ANOVA or Wald Chi-Square Test ($p<0.05$).

[d] Significant Levene's Test for homogeneity of variances ($p < 0.05$).

*Significantly different from control by Dunnett's Test or individual t-test ($p<0.05$).

Table 2-C. Vaginal Opening and Body Weight in Methoxychlor-Treated F1 Females [a]

Parameter	Methoxychlor (mg/kg/day, po)		
	0	25	50
Age at VO (days)	32.9 [b, c, d]	29.8 *	27.4 *
	± 0.4	± 0.6	± 0.3
BW at VO (grams)	127.89 [b, c, d]	104.85 *	89.54 *
	± 3.20	± 4.13	± 2.40
Initial BW (pnd 22, grams)	58.42	58.73	58.44
	± 0.97	± 1.08	± 1.21
Final BW (pnd 42, grams)	183.39 [b, c]	174.38	171.62 *
	± 3.61	± 2.87	± 2.59
Final BW as % of control (pnd 42)		95.1	93.6
BW gain (pnd 22 to 42, grams)	124.97 [b, c]	115.57 *	113.18 *
	± 3.00	± 2.17	± 2.57

[a] Mean ± SE (n = 15 for each treatment group).

[b] Significant Test for Linear Trend ($p<0.05$).

[c] Significant treatment effect by ANOVA or Wald Chi-Square Test ($p<0.05$).

[d] Significant Levene's Test for homogeneity of variances ($p < 0.05$).

*Significantly different from control by Dunnett's Test or individual t-test ($p<0.05$).

Table 2-D. Vaginal Opening and Body Weight in Bisphenol A-Treated F1 Females [a]

Parameter	Bisphenol A (mg/kg/day, po)		
	0	400	600
Age at VO (days)	32.3 ± 0.4	33.0 ± 0.6 (12)	32.3 ± 0.7 (14)
BW at VO (grams)	122.70[b, c] ± 3.17	116.36 ± 3.50 (12)	180.42 * ± 3.96 (14)
Initial BW (pnd 22, grams)	59.20 ± 0.81	58.48 ± 0.97	59.32 ± 0.82
Final BW (pnd 42, grams)	180.68 [b, c] ± 2.84 (14)	160.21 * ± 2.38 (11)	155.05 * ± 2.91 (14)
Final BW as % of control (pnd 42)		88.7 (11)	85.8 (14)
BW gain (pnd 22 to 42, grams)	121.11 [b, c] ± 2.79 (14)	101.82 * ± 1.45 (11)	95.86 * ± 2.50 (14)

[a] Mean ± SE (n = 15 for each treatment group unless otherwise noted in parentheses).

[b] Significant Test for Linear Trend (p<0.05).

[c] Significant treatment effect by ANOVA (p<0.05).

*Significantly different from control by Dunnett's Test (p<0.05).

Table 2-E. Vaginal Opening and Body Weight in Ketoconazole-Treated F1 Females [a]

Parameter	Ketoconazole (mg/kg/day, po)		
	0	50	100
Age at VO (days)	32.3 ± 0.4	33.0 ± 0.4	33.7 ± 0.6
BW at VO (grams)	122.70 ± 3.17	126.54 ± 2.54	122.60 ± 3.65
Initial BW (pnd 22, grams)	59.20 ± 0.81	59.58 ± 1.21	58.96 ± 0.96
Final BW (pnd 42, grams)	180.68 [b, c] ± 2.84 (14)	181.12 ± 2.99	166.39 * ± 2.96
Final BW as % of control (pnd 42)		100.2	92.1
BW gain (pnd 22 to 42, grams)	121.11 [b, c] ± 2.79 (14)	121.54 ± 2.11	107.42 * ± 2.54

[a] Mean ± SE (n = 15 for each treatment group unless otherwise noted in parentheses).

[b] Significant Test for Linear Trend ($p<0.05$).

[c] Significant treatment effect by ANOVA ($p<0.05$).

*Significantly different from control by Dunnett's Test ($p<0.05$).

Table 2-F. Vaginal Opening and Body Weight in Propylthiouracil-Treated F1 Females [a]

Parameter	Propylthiouracil (mg/kg/day, po)		
	0	2	25
Age at VO (days)	32.3 ± 0.4	33.3 ± 0.5	33.1 ± 0.4
BW at VO (grams)	122.70 [b, c] ± 3.17	127.35 ± 2.53	117.71 ± 1.86
Initial BW (pnd 22, grams)	59.20 ± 0.81	58.84 ± 0.94	59.54 ± 0.87
Final BW (pnd 42, grams)	180.68 [b, c] ± 2.84 (14)	175.77 ± 2.73	130.75 * ± 2.57
Final BW as % of control (pnd 42)		97.3	72.4
BW gain (pnd 22 to 42, grams)	121.11 [b, c] ± 2.79 (14)	116.93 ± 2.82	71.21 * ± 2.18

[a] Mean ± SE (n = 15 for each treatment group unless otherwise noted in parentheses).

[b] Significant Test for Linear Trend ($p<0.05$).

[c] Significant treatment effect by ANOVA ($p<0.05$).

*Significantly different from control by Dunnett's Test ($p<0.05$).

Table 3-A. Necropsy and Hormone Data for the Atrazine-Treated F_1 Females (page 1 of 2)

	Atrazine (mg/kg/day, po)		
	0	**75**	**150**
Sacrifice Body Weight (g)[a]	184.55[c, d] ± 4.05	169.84 * ± 2.87	159.01* ± 3.39
Pituitary Gland Weight (g)[a]	0.0105 [c, d] ± 0.0004	0.0099 ± 0.0007 (14)	0.0083 * ± 0.0003 (14)
Thyroid Gland Weight (g)[a]	0.0193 ± 0.0021 (14)	0.0162 ± 0.0010	0.0161 ± 0.0012
Liver Weight (g)[a]	9.7068 [c] ± 0.3705	9.0085 ± 0.2131	8.7233 ± 0.3560
Paired Adrenal Gland Weight (g)[a]	0.0432 ± 0.0027	0.0478 ± 0.0022	0.0414 ± 0.0021
Paired Kidney Weight (g)[a]	1.8590 ± 0.0490	1.8054 ± 0.0355	1.7377 ± 0.0494 (14)
Paired Ovary Weight (g)[a]	0.1015 [c, d] ± 0.0046	0.0962 ± 0.0040	0.0823 * ± 0.0044
Uterus with Fluid Weight (g)[a]	.3694 ± 0.0285	0.3568 ± 0.0345	0.3215 ± 0.0251
Uterus without Fluid Weight (g)[a]	0.3433 ± 0.0186	0.3299 ± 0.0223	0.2965 ± 0.0221
Adjusted Pituitary Gland Weight (g)[b]	0.0102 ± 0.0006	0.0099 ± 0.0005 (14)	0.0085 ± 0.0006 (14)
Adjusted Thyroid Gland Weight (g)[b]	0.0200 ± 0.0017 (14)	0.0162 ± 0.0015	0.0155 ± 0.0016

(continued)

Table 3-A. Necropsy and Hormone Data for the Atrazine-Treated F1 Females (page 2 of 2)

	Atrazine (mg/kg/day, po)		
	0	75	150
Adjusted Liver Weight (g)[b]	8.5691[c, d] ± 0.1515	9.1185 * ± 0.1303	9.7510 * ± 0.1478
Adjusted Paired Adrenal Gland Weight (g)[b]	0.0426 ± 0.0028	0.0478 ± 0.0024	0.0419 ± 0.0027
Adjusted Paired Kidney Weight (g)[b]	1.7230 [c, d] ± 0.0318	1.8187 ± 0.0273	1.8691 * ± 0.0324 (14)
Adjusted Paired Ovary Weight (g)[b]	0.1009 [c] ± 0.0051	0.0962 ± 0.0044	0.0829 ± 0.0050
Adjusted Uterus with Fluid Weight (g)[b]	0.3644 ± 0.0349	0.3573 ± 0.0300	0.3261 ± 0.0340
Adjusted Uterus without Fluid Weight (g)[b]	0.3361 ± 0.0247	0.3306 ± 0.0213	0.3030 ± 0.0241
Thyroxine Hormone (T4) (ug/dL)[a]	4.44 ± 0.24	4.55 ± 0.21	4.67 ± 0.24
Thyroid Stimulating Hormone (TSH) (ng/ml)[a]	9.62 ± 0.53	9.90 ± 0.79	8.52 ± 0.63
Endocrine Status[e]			
Diestrus	2	2	3
Proestrus/Estrus	6	7	6
Not cycling	2	1	1

[a] Reported as the mean + S.E.M.; pnd = postnatal day. n = 15 unless otherwise noted in parentheses.

[b] Reported as the adjusted mean + S.E.M. (sacrifice weight as covariate). n = 15 unless otherwise noted in parentheses.

[c] Significant Test for Linear Trend Test or Linear Trend of Covariance ($p<0.05$).

[d] Significant treatment effect by ANOVA or ANCOVA ($p<0.05$).

[e] Number of females; last vaginal smear before necropsy.

* Significantly different from control by Dunnett's Test or Dunnett's Test ($p<0.05$).

Table 3-B. Necropsy and Hormone Data for the Fenarimol-Treated F_1 Females (page 1 of 2)

	Fenarimol (mg/kg/day, po)		
	0	**50**	**250**
Sacrifice Body Weight (g)[a]	184.55[c,d] ±4.05	185.94 ±3.43	166.54* ±3.91
Pituitary Gland Weight (g)[a]	0.0105[c] 0.0004	0.0105 0.0005	0.0087***** 0.0002
Thyroid Gland Weight (g)[a]	0.0193 ±0.0021 (14)	0.0154 ±0.0011	0.0151 ±0.0009
Liver Weight (g)[a]	9.7068[c,d] ±0.3705	11.4820 ±0.3813	14.1926***** ±0.8964
Paired Adrenal Gland Weight (g)[a]	0.0432 ±0.0027	0.0524 ±0.0030	0.0441 ±0.0028
Paired Kidney Weight (g)[a]	1.8590[c] ±0.0490	1.9687 ±0.0528	1.7640 ±0.0435
Paired Ovary Weight (g)[a]	0.1015[c] ±0.0046	0.1106 ±0.0051	0.0895 ±0.0044
Uterus with Fluid Weight (g)[a]	0.3694 ±0.0285	0.3729 ±0.0255	0.3118 ±0.0317
Uterus without Fluid Weight (g)[a]	0.3433[c,d] ±0.0186	0.3526 ±0.0201	0.2782***** ±0.0187
Adjusted Pituitary Gland Weight (g)[b]	0.0105[c,d] ±0.0004	0.0105 ±0.0004	0.0088***** ±0.0004
Adjusted Thyroid Gland Weight (g)[b]	0.0189 ±0.0015	0.0147 ±0.0014	0.0161 ±0.0015 (14)
Adjusted Liver Weight (g)[b]	9.1071[c,d] ±0.4551	10.7322***** ±0.4612	15.5422* ±0.4975

(continued)

Table 3-B. Necropsy and Hormone Data for the Fenarimol-Treated F_1 Females (page 2 of 2)

	Fenarimol (mg/kg/day, po)		
	0	50	250
Adjusted Paired Adrenal Gland Weight (g)[b]	0.0427 ±0.0029	0.0518 ±0.0030	0.0452 ±0.0032
Adjusted Paired Kidney Weight (g)[b]	1.7999[d] ±0.0278	1.8949***** ±0.0282	1.8968 ±0.0304
Adjusted Paired Ovary Weight(g)[b]	0.1021[c] ±0.0049	0.1113 ±0.0050	0.0883 ±0.0053
Adjusted Uterus with Fluid Weight (g)[b]	0.3712 ±0.0297	0.3752 ±0.0301	0.3077 ±0.0325
Adjusted Uterus without Fluid Weight (g)[b]	0.3435[c] ±0.0199	0.3529 ±0.0201	0.2776 ±0.0217
Thyroxine Hormone (T4) (ug/dL)[a]	4.44[c,d] ±0.24	4.72 ±0.24	3.73***** ±0.13
Thyroid Stimulating Hormone (TSH) (ng/ml)[a]	9.62[c,d,e] ±0.53	12.53***** ±0.81	19.65***** ±2.29
Endocrine Status[f]			
Diestrus	2	3	4
Proestrus/Estrus	6	7	5
Not cycling	2	2	3

[a] Reported as the mean ± S.E.M.; pnd = postnatal day. n = 15 unless otherwise noted in parentheses.
[b] Reported as the adjusted mean ± S.E.M. (sacrifice weight as covariate). n = 15 unless otherwise noted in parentheses.
[c] Significant Test for Linear Trend Test or Linear Trend of Covariance ($p<0.05$).
[d] Significant treatment effect by ANOVA, ANCOVA or Wald Chi-Square Test ($p<0.05$).
[e] Significant Levene's test for homogeneity of variances ($p<0.05$).
[f] Number of females; last vaginal smear before necropsy.
* Significantly different from control by Dunnett's Test or individual t-test ($p<0.05$).

Table 3-C. Necropsy and Hormone Data for the Methoxychlor-Treated F_1 Females (page 1 of 3)

	Methoxychlor (mg/kg/day, po)		
	0	25	50
Sacrifice Body Weight (g)[a]	184.55 [c, d] ± 4.05	175.02 ± 3.13 (14)	172.81 * ± 2.85
Pituitary Gland Weight (g)[a]	0.0105 ± 0.0004	0.0097 ± 0.0004 (14)	0.0094 ± 0.0005 (13)
Thyroid Gland Weight (g)[a]	0.0193 ± 0.0021 (14)	0.0149 ± 0.0007 (14)	0.0168 ± 0.0008
Liver Weight (g)[a]	9.7068 ± 0.3705	8.8628 ± 0.2840 (14)	8.8458 ± 0.2485
Paired Adrenal Gland Weight (g)[a]	0.0432 ± 0.0027	0.0439 ± 0.0030 (14)	0.0463 ± 0.0030
Paired Kidney Weight (g)[a]	1.8590 ± 0.0490	1.8244 ± 0.0506 (14)	1.7544 ± 0.0511
Paired Ovary Weight (g)[a]	0.1015 [c] ± 0.0046	0.0916 ± 0.0041 (14)	0.0867 ± 0.0049
Uterus with Fluid Weight (g)[a]	0.3694 ± 0.0285	0.4092 ± 0.0467 (14)	0.4645 ± 0.0615
Uterus without Fluid Weight (g)[a]	0.3433 ± 0.0186	0.3504 ± 0.0243 (14)	0.3652 ± 0.0267

(continued)

Table 3-C. Necropsy and Hormone Data for the Methoxychlor-Treated F_1 Females (page 2 of 3)

	Methoxychlor (mg/kg/day, po)		
	0	25	50
Adjusted Pituitary Gland Weight (g)[b]	0.0103 ± 0.0004	0.0098 ± 0.0004 (14)	0.0095 ± 0.0005 (13)
Adjusted Thyroid Gland Weight (g)[b]	0.0190 ± 0.0014 (14)	0.0149 ± 0.0014 (14)	0.0170 ± 0.0013
Adjusted Liver Weight (g)[b]	9.1354 ± 0.1406	9.0659 ± 0.1392 (14)	9.2277 ± 0.1368
Adjusted Paired Adrenal Gland Weight (g)[b]	0.0434 ± 0.0030	0.0438 ± 0.0030 (14)	0.0462 ± 0.0030
Adjusted Paired Kidney Weight (g)[b]	1.7830 ± 0.0362	1.8514 ± 0.0358 (14)	1.8052 ± 0.0352
Adjusted Paired Ovary Weight (g)[b]	0.1029 [c, d] ± 0.0047	0.0911 ± 0.0047 (14)	0.0857 ***** ± 0.0046
Adjusted Uterus with Fluid Weight (g)[b]	0.3865 ± 0.0494	0.4032 ± 0.0489 (14)	0.4531 ± 0.0480
Adjusted Uterus without Fluid Weight (g)[b]	0.3534 ± 0.0241	0.3468 ± 0.0239 (14)	0.3584 ± 0.0234
Thyroxine Hormone (T4) (ug/dL)[a]	4.44 ± 0.24	4.57 ± 0.15 (14)	4.69 ± 0.17
Thyroid Stimulating Hormone (TSH) (ng/ml)[a]	9.62 ± 0.53	8.34 ± 0.47 (14)	9.83 ± 0.98

(continued)

Table 3-C. Necropsy and Hormone Data for the Methoxychlor-Treated F_1 Females (page 3 of 3)

	Methoxychlor (mg/kg/day, po)		
	0	25	50
Endocrine Status[e]			
Diestrus	2	2	3
Proestrus/Estrus	6	8	7
Not cycling	2	0	3

[a] Reported as the mean ± S.E.M.; pnd = postnatal day. n = 15 unless otherwise noted in parentheses.
[b] Reported as the adjusted mean ± S.E.M. (sacrifice weight as covariate). n = 15 unless otherwise noted in parentheses.
[c] Significant Test for Linear Trend or Linear Trend of Covariance($p<0.05$).
[d] Significant treatment effect by ANOVA ($p<0.05$).
[e] Number of females; last vaginal smear before necropsy.
* Significantly different from control by Dunnett's Test ($p<0.05$).

Table 3-D. Necropsy and Hormone Data for the Bisphenol A-Treated F_1 Females (page 1 of 3)

	Bisphenol A (mg/kg/day, po)		
	0	**400**	**600**
Sacrifice Body Weight (g)[a]	180.17 [c, d] ± 3.11 (14)	160.62 ** ± 2.76 (11)	155.27 ** ± 3.13 (14)
Pituitary Gland Weight (g)[a]	0.0087 ± 0.0009 (14)	0.0076 ± 0.0006 (10)	0.0075 ± 0.0007 (14)
Thyroid Gland Weight (g)[a]	0.0143 ± 0.0008 (13)	0.0126 ± 0.0005 (11)	0.0134 ± 0.0007 (14)
Liver Weight (g)[a]	9.7498 [c, d] ± 0.3130 (14)	8.1129 ** ± 0.3039 (11)	7.8492 ** ± 0.2914 (14)
Paired Adrenal Gland Weight (g)[a]	0.0478 [c] ± 0.0029 (13)	0.0414 ± 0.0019 (11)	0.0412 ± 0.0016 (14)
Paired Kidney Weight (g)[a]	1.8109 [c, d] ± 0.0530 (14)	1.6321 ** ± 0.0432 (11)	1.5900 ** ± 0.0489 (14)
Paired Ovary Weight (g)[a]	0.1009 [c, d] ± 0.0048 (14)	0.0734 ** ± 0.0050 (11)	0.0663 ** ± 0.0048 (14)
Uterus with Fluid Weight (g)[a]	0.3276 [c] ± 0.0261 (14)	0.2695 ± 0.0212 (11)	0.2436 ± 0.0293 (14)
Uterus without Fluid Weight (g)[a]	0.3057 [c, d] ± 0.0255 (14)	0.2532 ± 0.0214 (11)	0.2080 ** ± 0.0203 (14)
Adjusted Pituitary Gland Weight (g)[b]	0.0071 ± 0.0008 (14)	0.0082 ± 0.0008 (10)	0.0087 ± 0.0007 (14)

(continued)

Table 3-D. Necropsy and Hormone Data for the Bisphenol-Treated F_1 Females (page 2 of 3)

	Bisphenol A (mg/kg/day, po)		
	0	400	600
Adjusted Thyroid Gland Weight (g)[b]	0.0136 ± 0.0008 (13)	0.0128 ± 0.0007 (11)	0.0139 ± 0.0007 (14)
Adjusted Liver Weight (g)[b]	8.4459 ± 0.1628 (14)	8.5728 ± 0.1468 (11)	8.7918 ± 0.1464 (14)
Adjusted Paired Adrenal Gland Weight (g)[b]	0.0419 ± 0.0025 (13)	0.0433 ± 0.0021 (11)	0.0452 ± 0.0021 (14)
Adjusted Paired Kidney Weight (g)[b]	1.6342 ± 0.0411 (14)	1.6945 ± 0.0370 (11)	1.7177 ± 0.0369 (14)
Adjusted Paired Ovary Weight (g)[b]	0.0917 [c] ± 0.0057 (14)	0.0766 ± 0.0051 (11)	0.0729 ± 0.0051 (14)
Adjusted Uterus with Fluid Weight (g)[b]	0.3012 ± 0.0327 (14)	0.2788 ± 0.0295 (11)	0.2627 ± 0.0294 (14)
Adjusted Uterus without Fluid Weight (g)[b]	0.2768 ± 0.0278 (14)	0.2634 ± 0.0250 (11)	0.2290 ± 0.0250 (14)
Thyroxine Hormone (T4) (ug/dL)[a]	4.81 ± 0.27 (14)	4.88 ± 0.24 (11)	5.23 ± 0.24 (14)
Thyroid Stimulating Hormone (TSH) (ng/mL)[a]	7.88 ± 0.41	6.97 ± 0.30	7.51 ± 0.42 (14)

(continued)

Table 3-D. Necropsy and Hormone Data for the Bisphenol-Treated F_1 Females (page 3 of 3)

	Bisphenol A (mg/kg/day, po)		
	0	**400**	**600**
Endocrine Status[f]			
Diestrus	6	8	1
Proestrus/Estrus	4	3	4
Not cycling	3	1	3

[a] Reported as the mean ± S.E.M.; pnd = postnatal day. n = 15 unless otherwise noted in parentheses.
[b] Reported as the adjusted mean ± S.E.M. (sacrifice weight as covariate). n = 15 unless otherwise noted in parentheses.
[c] Significant Test for Linear Trend Test or Linear Trend of Covariance ($p<0.05$).
[d] Significant treatment effect by ANOVA or ANCOVA ($p<0.05$) or Wald Chi-Square Test.
[e] Significant Levene's test for homogeneity of variances ($p<0.05$).
[f] Number of females; last vaginal smear before necropsy.
* Significantly different from control by Dunnett's Test or individual t-test ($p<0.05$).

Table 3-E. Necropsy and Hormone Data for the Ketoconazole-Treated F_1 Females (page 1 of 3)

	Ketoconazole (mg/kg/day, po)		
	0	**50**	**100**
No. of Females on Study	15	15	15
Sacrifice Body Weight (g)[a]	180.17 [c, d] ± 3.11 (14)	178.34 ± 3.27	166.37 * ± 3.52
Pituitary Gland Weight (g)[a]	0.0087 [e] + 0.0009 (14)	0.0088 ± 0.0005 (14)	0.0080 ± 0.0004
Thyroid Gland Weight (g)[a]	0.0143 + 0.0008 (13)	0.0147 ± 0.0005	0.0144 ± 0.0007
Liver Weight (g)[a]	9.7498 ± 0.3130 (14)	10.7657 ± 0.2997	10.5637 ± 0.3982
Paired Adrenal Gand Weight (g)[a]	0.0478[c,d] ± 0.0029 (13)	0.0799* ± 0.0039	0.0785* ± 0.0037 (14)
Paired Kidney Weight (g)[a]	1.8109 [c] ± 0.0530 (14)	1.9571 ±0.0519	1.9827 ± 0.0581
Paired Ovary Weight (g)[a]	0.1009 [c, d] ± 0.0048 (14)	0.0968 ± 0.0051 (14)	0.0843 * ± 0.0037
Uterus with Fluid Weight (g)[a]	0.3276 [c, d] ± 0.0261 (14)	0.2981 ± 0.0239	0.2390 * ± 0.0158
Uterus without Fluid Weight (g)[a]	0.3057 [c, d] + 0.0255 (14)	0.2730 ± 0.0172	0.2217 * ± 0.0152

(continued)

Table 3-E. Necropsy and Hormone Data for the Ketoconazole-Treated F_1 Females (page 2 of 3)

	Ketoconazole (mg/kg/day, po)		
	0	**50**	**100**
Adjusted Pituitary Gland Weight (g)[b]	0.0084 ± 0.0008 (14)	0.0085 ± 0.0005 (14)	0.0085 ± 0.0005
Adjusted Thyroid Gland Weight (g)[b]	0.0140 ± 0.0007 (13)	0.0145 ± 0.0006	0.0149 ± 0.0007
Adjusted Liver Weight (g)[b]	9.2651 [c, d] ± 0.1696 (14)	10.4472 ** ± 0.1613	11.3346 ** ± 0.1716
Adjusted Paired Adrenal Gland Weight (g)[b]	0.0048[c,d] 0.0035 (13)	0.0781* 0.0032	0.0833* 0.0036 (14)
Adjusted Paired Kidney Weight (g)[b]	1.7423 [c, d] ± 0.0361 (14)	1.9120 * ± 0.0343	2.0919 ** ± 0.0365
Adjusted Paired Ovary Weight (g)[b]	0.0983 ± 0.0044 (14)	0.0948 ± 0.0044 (14)	0.0886 ± 0.0045
Adjusted Uterus with Fluid Weight (g)[b]	0.3182 ± 0.0228 (14)	0.2920 ± 0.0217	0.2540 ± 0.0231
Adjusted Uterus without Fluid Weight (g)[b]	0.2969 [c] ± 0.0200 (14)	0.2672 ± 0.0190	0.2357 ± 0.0202
Thyroxine Hormone (T4) (ug/dL)[a]	4.81 ± 0.27 (14)	4.97 ± 0.16	4.79 ± 0.19

(continued)

Table 3-E. Necropsy and Hormone Data for the Ketoconazole-Treated F_1 Females (page 3 of 3)

	Ketoconazole (mg/kg/day, po)		
	0	**50**	**100**
Thyroid Stimulating Hormone (TSH) (ng/ml)[a]	7.88 ± 0.41 (14)	8.91 ± 0.82	8.24 ± 0.47 (14)
Endocrine Status[f]			
Diestrus	6	5	6
Proestrus/Estrus	4	5	2
Not cycling	3	1	0

[a] Reported as the mean ± S.E.M.; pnd = postnatal day. n = 15 unless otherwise noted in parentheses.

[b] Reported as the adjusted mean ± S.E.M. (sacrifice weight as covariate). n = 15 unless otherwise noted in parentheses.

[c] Significant Test for Linear Trend Test or Linear Trend Analysis of Covariance ($p<0.05$).

[d] Significant treatment effect by ANOVA ($p<0.05$).

[e] Significant Levene's test for homogeneity of variances ($p<0.05$).

[f] Number of females; last vaginal smear before necropsy.

* Significantly different from control by Dunnett's Test ($p<0.05$).

Table 3-F. Necropsy and Hormone Data for the Propylthiouracil-Treated F_1 Females (page 1 of 3)

	Propylthiouracil (mg/kg/day, po)		
	0	2	25
Sacrifice Body Weight (g)[a]	180.17 [c, d] ± 3.11 (14)	75.25 ± 2.50	130.32 * ± 2.60
Pituitary Gland Weight (g)[a]	0.0087 ± 0.0009 (14)	0.0085 ± 0.0006	0.0075 ± 0.0005
Thyroid Gland Weight (g)[a]	0.0143 [c, d, e] ± 0.0008 (13)	0.0385 * ± 0.0016	0.0620 * ± 0.0048
Liver Weight (g)[a]	9.7498 [c, d] ± 0.3130 (14)	9.0381 ± 0.2596	5.8020 ***** ± 0.2095
Paired Adrenal Gland Weight (g)[a]	0.0478 [c, d] ± 0.0029 (13)	0.0420 ± 0.0020	0.0277 ***** ± 0.0010 (13)
Paired Kidney Weight (g)[a]	1.8109 [c, d] ± 0.0530 (14)	1.7156 ± 0.0420	1.2506 ***** ± 0.0942
Paired Ovary Weight (g)[a]	0.1009 [c, d] ± 0.0048 (14)	0.0893 ± 0.0037	0.0756 * ± 0.0037
Uterus with Fluid Weight (g)[a]	0.3276 ± 0.0261 (14)	0.3449 ± 0.0270	0.3408 ± 0.0363
Uterus without Fluid Weight (g)[a]	0.3057 ± 0.0255 (14)	0.3192 ± 0.0229	0.3015 ± 0.0208
Adjusted Pituitary Gland Weight (g)[b]	0.0071 ± 0.0009 (14)	0.0073 ± 0.0008	0.0102 ± 0.0013

(continued)

Table 3-F. Necropsy and Hormone Data for the Propylthiouracil-Treated F_1 Females (page 2 of 3)

	Propylthiouracil (mg/kg/day, po)		
	0	2	25
Adjusted Thyroid Gland Weight (g)[b]	0.0077 [c, d, e] ± 0.0041 (13)	0.0334 * ± 0.0035	0.0728 * ± 0.0096
Adjusted Liver Weight (g)[b]	8.2414 ± 0.2103 (14)	7.9265 ± 0.1797	8.3215 ± 0.2898
Adjusted Paired Adrenal Gland Weight (g)[b]	0.0449 ± 0.0032 (13)	0.0399 ± 0.0026	0.0330 ± 0.0049 (13)
Adjusted Paired Kidney Weight (g)[b]	1.6368 ± 0.0937 (14)	1.5873 ± 0.0800	1.5414 ± 0.1291
Adjusted Paired Ovary Weight (g)[b]	0.0907 ± 0.0056 (14)	0.0817 ± 0.0048	0.0926 ± 0.0078
Adjusted Uterus with Fluid Weight (g)[b]	0.3288 ± 0.0454 (14)	0.3458 ± 0.0388	0.3389 ± 0.0626
Adjusted Uterus without Fluid Weight (g)[b]	0.2860 ± 0.0342 (14)	0.3047 ± 0.0292	0.3343 ± 0.0472
Thyroxine Hormone (T4) (ug/dL)[a]	4.81 [c, d, e] ± 0.27 (14)	1.97 * ± 0.27 (14)	0.66 * ± 0.02 (2)

(continued)

Table 3-F. Necropsy and Hormone Data for the Propylthiouracil-Treated F_1 Females (page 3 of 3)

	Propylthiouracil (mg/kg/day, po)		
	0	**2**	**25**
Thyroid Stimulating Hormone (TSH) (ng/ml)[a]	7.88 [c, d, e] ± 0.41 (14)	42.36 * ± 6.14	99.41 * ± 5.67
Endocrine Status[f]			
Diestrus	6	6	5
Proestrus/Estrus	4	4	6
Not cycling	3	0	0

[a] Reported as the mean ± S.E.M.; pnd = postnatal day. n = 15 unless otherwise noted in parentheses.
[b] Reported as the adjusted mean ± S.E.M. (sacrifice weight as covariate). n = 15 unless otherwise noted in parentheses.
[c] Significant Test for Linear Trend Test or Linear Trend Analysis of Covariance ($p<0.05$).
[d] Significant treatment effect by ANOVA, ANCOVA or Wald Chi-Square Test ($p<0.05$).
[e] Significant Levene's test for homogeneity ($p<0.05$).
[f] Number of females; last vaginal smear before necropsy.
* Significantly different from control by Dunnett's Test or individual t-test ($p<0.05$).